THE VITAMIN G CONTENT OF HOME-CANNED TOMATO JUICE

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ONE FIGURE

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There seems to be some disagreement as to the vitamin G content of tomatoes. Hartley ('30) states that tomatoes contain about 0.16 unit of this vitamin; while, on the other hand, Daggs and Eaton ('32) claim that this vitamin is not present in the juice of the tomato. Goldberger and Wheeler ('27), in working with human beings, found tomatoes to possess well-marked pellagra-preventive action when fed in amounts of approximately 15 gm. per kilo of body weight.

The investigation reported in this paper gives the quantitative determination of the water soluble, growth-promoting factor other than vitamin B in home-canned tomato juice.

Ripe, sound tomatoes were gathered from the fields and immediately made into juice. The tomatoes were washed, and any adhering water was removed. They were then quartered, and all blemishes and soft spots were cut out. The tomatoes were crushed and placed in covered pans and were brought quickly to the boiling point. The juice was extracted by means of a rotary sieve, placed in quart jars, and sterilized in live steam for 30 minutes.

Healthy young albino rats, progeny of the Wistar strain, were weaned at 4 weeks and were given the following diet

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(Bourquin and Sherman, '31) which is deficient in vitamin G, but adequate in all other factors necessary for growth.

	Per cent
Purified casein	18
Butter fat, water and curd free	8
Cod liver oil	2
Salt mixture, Osborne and Mendel ('19)	4
Cornstarch	68

Alcoholic extract from 50 gm. of wheat for each 100 gm. of food mixture.

The animals were kept on the above diet until the surplus body store of vitamin G had been depleted as indicated by

TABLE 1

Table showing units of vitamin G in home-canned tomato juice

SAMPLE NO.	AMOUNT FED IN CUBIC CENTIMETERS	GAIN IN WEIGHT		UNITS	AVERAGE UNITS
		Total 8 weeks	Weekly average		
		gm.	*gm.*		
1	6	29	3.63	0.20	0.21
	8	43	5.38	0.22	
2	6	24	3.00	0.17	0.17
	8	33	4.13	0.17	
3	6	21	2.63	0.15	0.18
	8	38	4.75	0.20	
4	6	28	3.50	0.19	0.21
	8	44	5.50	0.23	
5	4	24	3.00	0.25	0.25
	6	34	4.25	0.24	
6	4	24	3.00	0.25	0.27
	6	41	5.13	0.29	
7	4	16	2.00	0.17	0.21
	6	34	4.25	0.24	
8	4	20	2.50	0.21	0.21
	6	29	3.63	0.20	
9	4	18	2.25	0.19	0.20
	6	30	3.75	0.21	
10	4	28	3.50	0.29	0.28
	6	37	4.63	0.26	
11	6	25	3.13	0.17	0.18
	8	35	4.38	0.18	
12	6	22	2.75	0.15	0.15
	8	29	3.63	0.15	
13	6	22	2.75	0.15	0.16
	8	33	4.13	0.17	
				Average	0.206

cessation of growth. The animals were then placed in separate metal cages with raised bottoms of wide-meshed wire screen so as to control coprophagy. Each animal was given a daily measured portion of tomato juice in addition to the basal diet, ad libitum.

The animals were weighed three times each week. The total gain in weight for an 8-week period was calculated for each animal. The weights of the animals which showed an average weekly gain nearest to 3 gm. were used to calculate

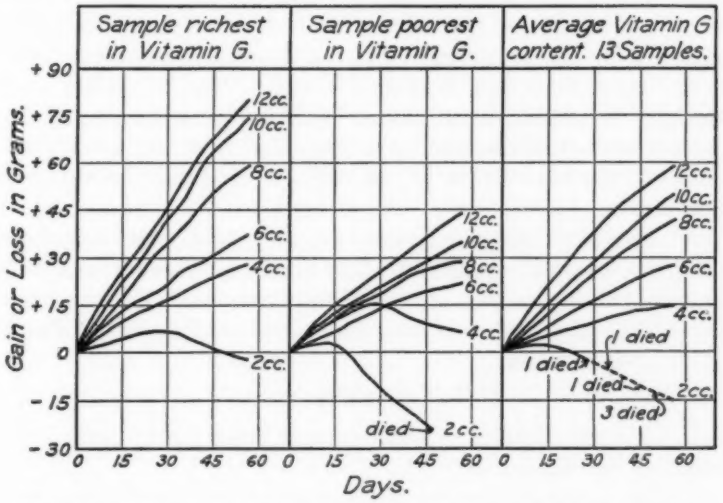

Fig. 1 Growth curves, showing the vitamin G content of tomato juices.

the Sherman units (Bourquin and Sherman, '31) present in each sample. These units are given in table 1.

Figure 1 shows the average growth curves for the animals fed the different samples, and also the growth curves for the animals fed the juices having the maximum and minimum amount of vitamin.

The vitamin content was also determined for the filtered juice, the first four samples being used. No appreciable amount of difference in vitamin content was noted over that found when the whole juice was fed.

It is evident from the data given in table 1 and figure 1 that all of the tomato juices tested contained a fair amount of a water-soluble factor other than vitamin B which promoted growth. Many authors accept this factor as vitamin G. However, as recently set forth in a review by Lewis ('33), vitamins B and G may contain a number of factors. Daggs and Eaton ('32) found a limited amount of a growth-promoting factor present in tomato juice, but stated that it could not be considered as vitamin G because they found definite evidence of dermatitis in feeding amounts of tomato juice up to 7 cc. It appears that these authors really tested their samples for the presence of the old vitamin B-complex, since their basal food was lacking in both vitamins B(B_1) and G(B_2). It seems to the present authors that the growth in the animals studied must have been due to vitamin G, since none of the rats fed 4 cc. or more of the juice developed dermatitis.

A relatively uniform amount of vitamin G was found in all of the samples examined. The minimum amount was 0.15 unit and the maximum amount was 0.28 unit. The average for all samples was 0.21 unit per cubic centimeter or about 95 units per pound.

SUMMARY

1. Thirteen samples of home-canned tomato juice have been assayed for vitamin G.

2. The average vitamin content per cubic centimeter has been found to be 0.21 unit.

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FURTHER STUDIES ON THE EFFECT OF COD LIVER OIL ON THE THYROID GLAND

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TWO PLATES (SIX FIGURES)

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It has previously been shown (Moore, '31) that the feeding of an excess of carotene to albino rats results in an accumulation in the liver of large amounts of vitamin A, and that an excess of carotene was found to persist apparently unchanged throughout the alimentary tract. Capper, McKibbin and Prentice ('31) have shown that the fowl as well as the rat can convert carotene into vitamin A. These investigators report that carotene is not stored in the liver unchanged, but is converted into vitamin A. It is obvious, therefore, that conversion to the vitamin takes place subsequent to absorption into the blood stream.

McCarrison ('19) reports that dietaries deficient in vitamins lead to a reduction in size and weight of the thyroid gland.

Kojima ('17) showed that the administration of sodium and potassium iodides caused an accumulation of colloid in the thyroid gland of rats. Chouke ('30) noted that intraperitoneal injection in daily doses of 0.005 gm. and 0.0025 gm. of potassium iodide in rats for periods of 10, 15, 20 and 30 days produced no appreciable change in the structure or in the character of the colloid of the thyroid gland.

Sherwood, Toth and Carr ('34) were able to produce marked changes in the thyroid gland of the rat after administering excessive amounts of cod liver oil.

It was decided, therefore, to investigate, as nearly as possible, the effects of some of the constituents of cod liver oil on the thyroid gland of the rat, and to confirm the results obtained by other investigators (Kojima, '17; Chouke, '30).

EXPERIMENTAL PROCEDURE

The experimental procedure was designed to determine the effect of vitamin A and iodine on the thyroid gland in approximately the same amount as found in cod liver oil. Albino rats received, in addition to our basal ration, excessive amounts of carotene, haliver oil, cod liver oil and potassium iodide.

Since carotene is converted into vitamin A (Moore, '31; Capper et al., '31) caritol (0.3 per cent carotene in oil) was used as a possible source for the vitamin investigation. To further observe the effects of vitamin A, haliver oil (Squibb) was used in one series of animals. Each sample of cod liver oil was analyzed for iodine content and approximately equivalent amounts of potassium iodide were given to another series of adult rats.

Group I. Fifteen animals were killed at regular intervals up to 29 days after having received 0.3 cc. haliver oil daily. This amount of haliver oil contained many times the amount of vitamin A found in the same dose of cod liver oil.

Group II. Seventeen animals were killed at regular intervals from 5 to 45 days after having received 0.1 cc. of caritol daily.

Group III. Six rats were used in order that we might confirm our previous report (Sherwood, Toth and Carr, '34). These animals were killed at 5-day intervals, the experiment covering a period of 30 days.

Group IV. Six rats received approximately the equivalent iodine found in 0.3 cc. of cod liver oil daily. Animals in this series were killed at regular intervals up to 33 days.

Control rats were kept under identical conditions, receiving the same basal diet as in the experimental groups. Control animals of the same age and sex as the experimental animals were killed for thyroid study in each group.

RESULTS

The control animals in each group show the typical normal thyroid gland with an even distribution of colloid and the normal epithelium lining the acini. Figure 1 indicates a normal rat thyroid.

The glands of the animals receiving haliver oil (fig. 2), caritol (fig. 3), and cod liver oil (fig. 4), show somewhat similar pictures, namely, an irregular distribution to a depletion of the colloid.

The cells lining the acini have increased in height and a marked increase in stroma has occurred. Occasionally phagocytic cells are found in the remaining colloid.

The series which received potassium iodide show an increase in colloid after 18 days of experimentation. In 33 days, the acini are strikingly distended, filled with non-vacuolated colloid and composed of extremely low epithelium. This is well demonstrated in figure 5.

DISCUSSION

Vitamin A has been shown to be vital to the growth of animals. It would, therefore, seem possible that some change would be produced in the glandular tissues of the body when diets are lacking in or over-loaded with vitamins. Agduhr ('29) showed that regressive tissue changes were produced with large doses of cod liver oil. The oestrous cycle was apparently affected, since his experimental animals showed no sign of heat.

Carotene and haliver oil have apparently altered the appearance of the thyroid gland, but failed to produce the marked results demonstrated in cod liver oil experiments. This would lead to the conclusion that vitamin A has some effect on the structure of the thyroid. Tissue growth is apparently introduced, since there is an increase of stroma. In the cod liver oil experiment, no increase of stroma is noted, but the epithelium of the acini is increased to more than twice the normal size and a colloid depletion takes place. In a previous report (Sherwood, Toth and Carr, '34) an excess of vitamin D was shown to have no effect on the thyroid gland.

It is of interest to note that the approximate amount of iodine as found in cod liver oil caused an increase in colloid. This is in agreement with the results obtained by Kojima ('17), but contrary to the results obtained by Chouke ('30). In the last mentioned reports far greater amounts of potassium iodide were used.

The iodine of cod liver oil is apparently not as readily available for the metabolic activities as it is in the form of potassium iodide. Much of the iodine of cod liver oil was lost, since the oil was excreted to some extent. This must have been the case, since the feces were very oily.

The minute amounts of iodine had no effect up to the eighteenth day, but following that period all animals showed a decided increase in thyroidal colloid. Apparently, an accumulation finally took place to produce the results obtained.

It has been suggested that the introduction of large amounts of oil might have caused a change in the thyroid structure and that the iodine might have been drawn out into the various fat reservoirs. However, animals receiving large doses of other oils do not show a colloid depletion.

SUMMARY AND CONCLUSIONS

Vitamin A apparently causes some change in the thyroid gland of rats.

Iodine present in the cod liver oil was not responsible for any change in the thyroid gland. However, iodine in the approximate amount found in cod liver oil, given in the form of potassium iodide had a decided effect on the thyroid structure of the rat.

A previous report of the senior author ('34) has been repeated and confirmed.

Due to the various results obtained, it is concluded that carotene and cod liver oil do not produce identical thyroid pictures, but in many respects these are similar.

We have pleasure in expressing our thanks to SMA Corporation, Cleveland, Ohio, for supplying the carotene used in the experiments; to E. R. Squibb & Sons for their generous supply of haliver oil; to Dr. J. S. McHargue for the iodine analyses of the cod liver oil samples; and to Dr. Alfred Brauer for his criticism of the manuscript.

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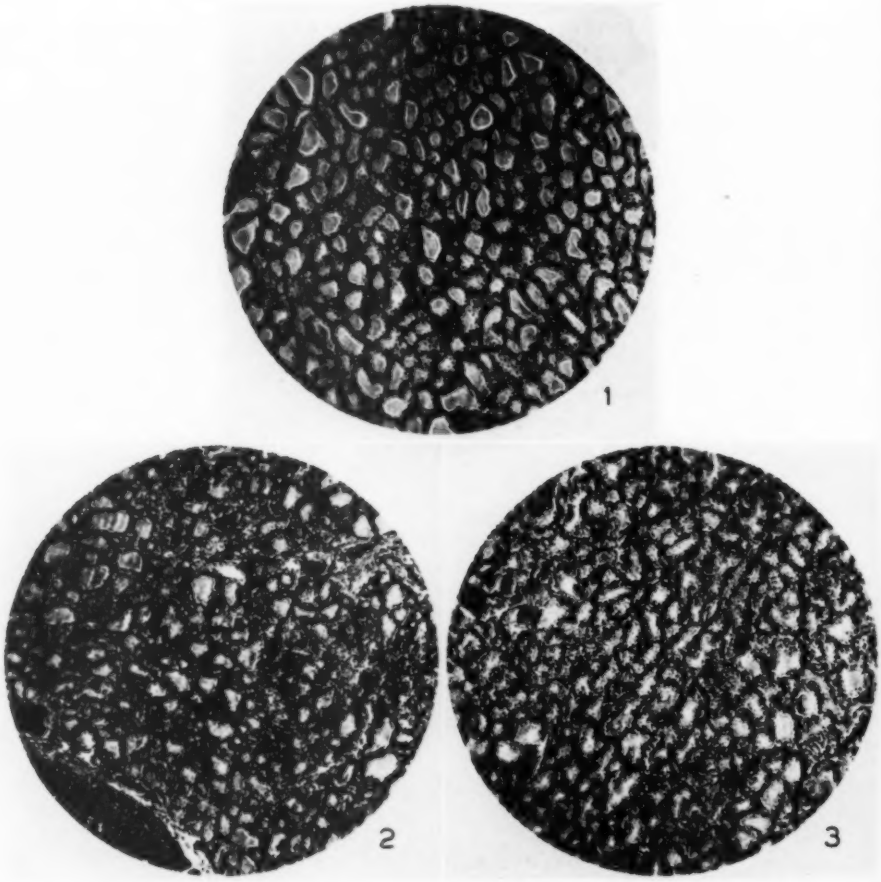
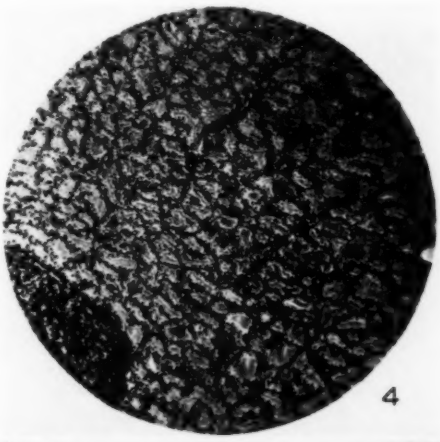
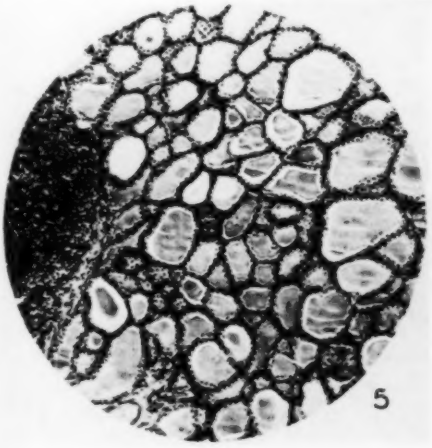


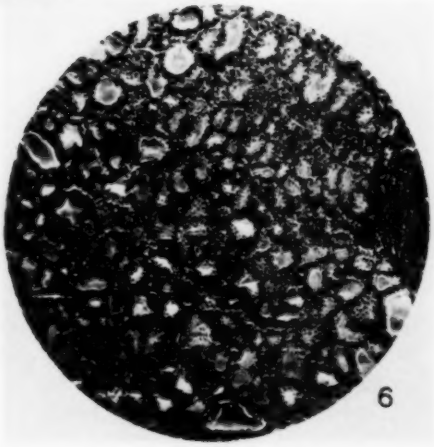
1 Photomierograph of thyroid section, showing normal gland. × 200 approx.

2 Photomierograph of thyroid section, showing effect of 21-day haliver oil experiment. × 200 approx.

3 Photomierograph of thyroid section, showing effect of 45-day carotene experimentation. × 200 approx.







4 Photomicrograph of thyroid section, showing effect of 28-day cod liver oil experimentation. × 200 approx.

5 Photomicrograph of thyroid section, showing effect of 33-day potassium iodide experiment. × 200 approx.

6 Photomicrograph of thyroid section, showing effect of 18-day cod liver oil experiment. × 200 approx.

EFFECTS OF LONG-CONTINUED CHOLESTEROL FEEDING IN RATS [1]

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TWO FIGURES

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Although cholesterol is a normal constituent of all animal food-stuffs, little attention has been paid to the effects on omnivorous animals of long-continued feeding of this sterol in amounts greater than are ordinarily present in the diet. It is well known that in herbivorous animals the ingestion of even small amounts of cholesterol causes a condition similar to arteriosclerosis in humans. It has not proved possible to reproduce this picture in omnivorous animals, but Yuasa ('28) and Schönheimer and Yuasa ('29) have shown that the cholesterol content of the liver increases markedly after long-continued cholesterol feeding in rats, mice and cats. This finding has been confirmed by Okey ('33), Chanutin and Ludewig ('33), and Blatherwick, Medlar, Bradshaw, Post and Sawyer ('33). Okey, Yokela and Knock ('34) have called attention to the possibility that a diet containing large amounts of cholesterol may have a detrimental effect in humans. They found that the cholesterol of the liver is markedly increased in rats after feeding dried egg yolk powder at a high level for about 2 months and conclude, "But egg yolk is being used extremely liberally in diabetic diets at the present time, and we feel that the high incidence of diseases associated with cholesterol deposition in diabetics (e.g., gall stones and

[1] This investigation was supported in part by the Josiah Macy, Jr., Foundation.

arteriosclerosis) may, at least, not be entirely unrelated to this fact."

In the present investigation we have studied long-continued cholesterol feeding in relation to growth, efficiency of food utilization, resistance to infection, and deposition of cholesterol in tissues.

I. CHOLESTEROL FEEDING IN RELATION TO GROWTH AND FOOD UTILIZATION[1]

The enormous deposits of cholesterol in the liver, which have been shown in previous and the present (p. 150) investigations to follow cholesterol feeding, might be expected to have an unfavorable influence on growth. The question has been studied in three series of experiments.

Series I was preliminary in nature. Control rats were placed at weaning on a diet composed of extracted casein, sugar, Crisco, salt mixture, Vitavose; and cod liver oil which was fed as a supplement. Casein was prepared according to the technic described by Sperry ('26). Analysis showed that it contained at the most only a trace of cholesterol. An equal number of rats received exactly the same diet, except for the addition of approximately 1.0 per cent of pure cholesterol. The cholesterol used in series I and II was prepared from gall stones by extraction with and several recrystallizations from alcohol. The solution was boiled with charcoal under reflux at the first recrystallization and the last recrystallization was from absolute alcohol. Litters were divided as equally as possible between the two groups and the sex ratio was the same in each.

At the time these experiments were carried out little information was available concerning the distribution of vitamin G and we were under the erroneous impression that Vitavose provided an ample supply of this factor. As was to have been expected, the rats did not grow well; after 162 days twelve rats on the low-cholesterol diet had an average weight

[1] A preliminary report of this part of the investigation was presented before the American Institute of Nutrition at New York, March 28, 1934.

of 142 gm., while an equal number on the cholesterol diet averaged 101 gm. The rats receiving cholesterol not only grew less well than the controls, but there was a marked difference in the general appearance of the two groups of animals. The controls, though small, looked perfectly normal. The cholesterol-fed rats, on the other hand, appeared grossly abnormal, as evidenced especially by roughness of coat and inactivity.

TABLE 1

Composition of diets—series II

SYNTHETIC DIETS			NATURAL-FOOD DIETS		
Constituent	Diet A	Diet B	Constituent	Diet C	Diet D
	gm.	gm.		gm.	gm.
Casein (extracted)	250	250	Oats	150	150
Crisco	200	200	Barley	150	150
Sugar	550	550	Wheat (spring)	150	150
Salt mixture[1]	50	50	Corn (yellow dent)	150	150
Vitavose	100	100	Soy bean meal	150	150
Vitamin Harris	10	10	Alfalfa meal	20	20
Spintrate	4	4	Casein (extracted)	200	200
Viosterol (cc.)	0.2	0.2	Salt mixture[1]	30	30
Cholesterol	0	10	Crisco	100	100
			Cholesterol	0	10

[1] McCollum and Simmonds ('18).

Series II. A group of rats of the Long-Evans strain was paired at weaning according to sex and in most instances according to litter. One-half of the pairs were placed upon synthetic diets A and B (table 1). One member of each pair was given the practically cholesterol-free diet A, while the other received exactly the same diet with cholesterol added(B). The other pairs were fed the natural-food diets C and D.[2] As in the case of the synthetic diets one member of each pair was placed on the cholesterol-free diet C, while the other was given exactly the same diet with cholesterol added (D). In both groups of cholesterol-fed rats analysis of the feces indicated that only a small part of the cholesterol was excreted.

[2] We are indebted to Dr. T. F. Zucker for suggesting the use of these diets.

The rats were housed in individual cages on wire screens and were fed from 'non-scattering' cups. A sheet of blotting paper was placed on the floor of each cage. The rats and the food consumed were weighed every 2 or 3 days. Any spilled food was collected carefully from the blotting papers.

Series III. In this experiment only male rats of the Wistar strain were used.[4] All rats were paired according to litter and in most instances there was little or no difference in the weights of the two rats in a pair. One rat in each pair was placed at weaning on diet A_1 and the other on diet B_1 which

TABLE 2

Composition of diets—series III

CONSTITUENT	DIET A_1	DIET B_1
	gm.	*gm.*
Casein (extracted)	200	200
Crisco	250	250
Sugar	365	365
Salt mixture[1]	50	50
Vitavose	100	100
Vitamin Harris	25	25
Spintrate	10	10
Viosterol (cc.)	0.3	0.3
Cholesterol	0	20

[1] Hawk and Oser ('31).

was exactly the same as A_1, except for the addition of cholesterol (table 2). Growth and food consumption were measured as in series II.

The cholesterol fed in this experiment was purified from a fraction obtained by chilling an alcoholic solution of the unsaponifiable material of cod liver oil, which was furnished through the courtesy of the National Oil Products Company, of Harrison, N. J. It was refluxed in alcohol in the presence of charcoal for many hours and recrystallized several times from alcohol and finally from ethylene chloride. The melting

[4] We express our gratitude to Dr. Erwin Brand and to Dr. C. J. Stucky, of the New York State Psychiatric Institute and Hospital, for careful selection of these rats from their colony.

point (corrected) was 148° to 149° and $[\alpha]_D^{20°}$ in ether was — 31.9°. The absence of appreciable amounts of ergosterol was demonstrated by ultraviolet spectrum analysis. During the first 6 weeks of the experiment the cholesterol which was used had also been purified through the bromide. This product had the same physical constants as the unbrominated cholesterol.

TABLE 3

Effect of dietary cholesterol on growth

AGE	NUMBER OF PAIRS	AVERAGE EXCESS WEIGHT OF RATS RECEIVING NO CHOLESTEROL	PROBABLE ERROR
Series II—Synthetic diet			
days		*gm.*	*gm.*
20	12	1.2	
40	12	10.3	2.7
60	12	21.4	4.9
80	12	26.8	5.1
100	11	22.4	2.9
Series III—Synthetic diet			
20	26	— 0.3	
40	24	6.6	2.8
60	18	14.3	4.1
80	18	18.7	4.5
100	14	29.1	5.5
120	8	31.4	9.1
Series II—Natural-food diet			
20	13	— 4.0	1.6
40	13	— 4.8	2.5
60	13	— 3.1	3.3
80	13	— 4.7	3.7
100	12	— 7.3	3.7

Growth. The growth curves were plotted for each rat, smoothed to rule out day-to-day variations, and the weights at 20, 40, 60, 80, 100 and 120 days were read off. The weights of the cholesterol-fed rats were subtracted from the corresponding weights of their paired controls, the differences were averaged, and the probable errors of the means were computed. The results are tabulated in table 3.

It is evident that rats on the synthetic diets without cholesterol grew better than their cholesterol-fed mates both in series II and III. The average excess weights were significant or nearly so for most of the ages measured after 20 days.

With the natural-food diets cholesterol showed no effect. Indeed, rats receiving cholesterol grew on the average a little better than the controls, but the differences are not significant.

The discrepancy between the findings with synthetic and natural-food diets is most probably related to the fact that the deposits of cholesterol esters found in the livers of rats which had received the natural-food diet with cholesterol averaged only about one-fifth as much as in rats which had received the synthetic diet. In this connection it is of interest that the highest values for cholesterol esters in the liver were found in the cholesterol-fed rats of series I, where the effect of cholesterol feeding on growth was most marked. We have no explanation for the smaller deposits of cholesterol esters on the natural-food diet. The finding that the combined cholesterol of the serum was much lower in cholesterol-fed rats on the natural-food diet than in the corresponding rats on the synthetic diet (table 10) might indicate that cholesterol was less well absorbed in the former, but no marked difference in absorption was indicated by fecal analysis.

Food consumption. The total weight of food (less its cholesterol content) consumed by each cholesterol-fed rat during the first 60 days on the diet was subtracted from the amount consumed by its paired control over the same period. The differences were averaged and the probable errors of the averages were calculated. Differences in food consumption during the first 99 days (series II) and the total period (series III) were determined in the same way. The data (table 4) show that rats on the synthetic diets without cholesterol consumed more food on the average than their controls. The differences were just significant for the 99-day period in series II and for the 60-day period in series III; they were almost significant for the remaining two periods. On the natural-food diet the cholesterol-fed rats ate more on the average than their controls, but the differences were not significant.

These results indicate that animals which grew faster also ate more food. The question arises whether the difference in growth was due entirely to the difference in food consumption or whether some other factor was concerned. Palmer and Kennedy ('31) proposed a formula for estimating the efficiency of food utilization:

$$\text{Efficiency quotient (E. Q.)} = \frac{\dfrac{\text{Digestible dry matter consumed}}{\text{Gain in weight}}}{\text{Mean weight during experiment}} \times 100$$

We felt that the calculation of efficiency quotients according to this formula might answer the above question. Such calculations have been made for the same periods as in the analysis

TABLE 4

Effect of dietary cholesterol on food consumption

SERIES	DIET	NUMBER OF PAIRS	PERIOD	AVERAGE EXCESS FOOD CONSUMPTION OF RATS RECEIVING NO CHOLESTEROL	PROBABLE ERROR
			days	*gm.*	*gm.*
II	Synthetic	12	60	45.7	14.5
II	Synthetic	11	99	75.5	18.2
III	Synthetic	21	60	31.0	7.8
III	Synthetic	26	Total[1]	35.2	10.1
II	Natural-food	13	60	— 33.0	13.9
II	Natural-food	12	99	— 59.0	21.9

[1] Varied from 27 to 138 days.

of food consumption. Digestible dry matter was not determined, since the diets compared were the same, except for cholesterol, for which correction was made. Total food consumed was substituted for 'digestible dry matter' in the formula. The difference in E. Q.'s was determined for each pair and the differences were averaged and treated statistically just as in the case of food consumption, except that the E.Q. of the rat which had not received cholesterol was subtracted from the E. Q. of the cholesterol-fed rat. It must be remembered that a higher E. Q. indicates less efficient utilization of food.

The results (table 5) show that for all periods analyzed on both the synthetic and natural-food diets the average E. Q.'s were higher for cholesterol-fed rats than for their controls. The differences were significant for all periods on the synthetic diets, but they were not nearly significant on the natural-food diet as was to have been expected from the inverse relationship found with this diet with respect to growth and food consumption.

The inclusion of the term 'mean weight during experiment' in the calculation of E.Q. had the effect of placing undue emphasis on the weight factor. In our experiments the gain

TABLE 5

Effect of dietary cholesterol on the efficiency of food utilization

SERIES	DIET	NUMBER OF PAIRS	PERIOD	AVERAGE DIFFERENCE IN E.Q.'S (E.Q. WITH CHOLESTEROL —E.Q. WITHOUT CHOLESTEROL)	AVERAGE DIFFERENCE IN FOOD CONSUMPTION PER GRAM GAIN
			days		
II	Synthetic	12	60	1.25 ± 0.28	0.53 ± 0.09
II	Synthetic	11	99	0.81 ± 0.16	0.34 ± 0.12
III	Synthetic	21	60	0.31 ± 0.06	0.19 ± 0.04
III	Synthetic	26	Total[1]	0.42 ± 0.07	0.27 ± 0.06
II	Natural-food	13	60	0.31 ± 0.25	0.33 ± 0.13
II	Natural-food	12	99	0.12 ± 0.20	0.37 ± 0.15

[1] Varied from 27 to 138 days.

in weight was approximately the same as the mean weight in many instances; consequently the weight factor entered the equation approximately squared. In other words, since in most of our experiments both rats in a pair weighed nearly the same at the start, the average weight was directly related to the gain in weight. Hence we have calculated the average food consumption per gram gain in weight (the upper term in Palmer and Kennedy's formula) for each of the rats and treated the data just as in the case of the E. Q.'s (table 5). The results agree with the E. Q.'s in indicating a decreased efficiency of food utilization in rats fed a cholesterol-containing synthetic diet.

II. CHOLESTEROL FEEDING IN RELATION TO RESISTANCE TO INFECTION[5]

There is considerable evidence indicating that cholesterol plays an active part in the defense mechanism of the organism. The literature on this subject has been reviewed recently by Degkwitz ('31) and need not be discussed in detail here. In general, it has been found that cholesterol protects red blood cells against hemolytic substances. This action has been well established in the case of saponins (Ransom, '01) with which cholesterol forms non-hemolytic combinations.

Numerous investigators have found that the total cholesterol of the blood is markedly reduced at the height of certain infections. (See Kipp, '20, and Shope, '30, for summaries of the literature; also see McQuarrie and Stoesser, '32.) It has been assumed by many that this decrease is due to a combination of cholesterol with bacterial toxins, similar to the combination with saponins, and Leupold and Bogendörfer ('22), Beumer ('23), Borchardt ('29), and Spranger (quoted by Degkwitz, '31, p. 835) have tested this hypothesis by comparing the resistance of cholesterol-fed and control animals to the same dose of bacterial suspension or toxin. They all found, in confirmation of the theory, that cholesterol-fed animals had greater resistance than controls, as shown by longer periods of survival,[6] and all concluded that cholesterol exerts a general protective action against bacterial infection. Borchardt gives no protocols; the other investigators used a total of thirty-four cholesterol-fed animals and twenty-eight controls. The diets are not described in detail by any of the authors. If the diets received by experimental and control animals differed in other respects than their cholesterol content, the effect may have been due to some other factor which was present in different amounts in the two diets.

[5] A preliminary report of this part of the investigation was presented before the Society for Pediatric Research at Atlantic City, May 1, 1934.

[6] Levinson ('31) found no effect of cholesterol feeding on resistance to tuberculosis in rabbits. Experiments in which cholesterol sols were injected, such as those of Lubinski and Stern ('26), are open to the criticism that the cholesterol in such emulsions is not in a physiological state. We have limited our discussion and experimental study to the effect of cholesterol feeding.

In view of the practical importance of the conclusion that cholesterol feeding has a general protective action against infection, the relatively small number of experiments on which the conclusion has been based, and the incomplete data concerning the diets, it seemed worth while to investigate the question further. This has been done by inoculating the rats described in part I with the paratyphoid organism Salmonella dansyz,[7] and comparing the survival of control and cholesterol-fed animals. The experiments were designed so that cholesterol might exert its maximum effect; control rats had received a diet containing at the most traces of cholesterol, while experimental rats had received exactly the same diet, except for the addition of cholesterol, for long periods of time (average period 141 days). Under these conditions, the cholesterol-fed rats might be expected to be saturated with cholesterol; that this was the case is shown by the analysis of tissues and blood (part III).

In all of the experiments both control and cholesterol-fed rats in each pair received the same amount of the same culture intraperitoneally. In order that the amount given might be lethal, but not large enough to overwhelm the animals, an attempt was made to determine the minimal lethal dose of the organism. This proved impossible, because the stock rats, which were used for the test, differed markedly in resistance from the rats which had been fed the experimental diets.[8]

We were forced to determine the proper dose by experience with the experimental animals themselves. This was done in series I and II by injecting a pair at a time and adjusting the dose given the next pair according to the results obtained with the first, etc. In most instances the periods of survival were satisfactory. In series III we extended this technic by adjusting the dose on the basis of bacterial counts of the cultures used.[9]

[7] We are indebted to Dr. Carl ten Broeck, of the Rockefeller Institute for Medical Research, Department of Animal and Plant Pathology, for suggesting the use of this paratyphoid organism, because of its general toxicity to rats.

[8] Dr. C. A. Slanetz furnished much valuable advice and assistance in making these tests and in preparing and injecting the cultures.

[9] Miss Florence Fitzpatrick kindly made these counts.

In series I the experiments were complicated by a probable vitamin G deficiency (p. 132). Despite a marked disadvantage in weight and condition, the cholesterol-fed rats showed considerably more resistance than their controls. Three cholesterol-fed rats recovered from the infection, while their much heavier controls, receiving the same amount of culture, died. The average survival of four other cholesterol-fed rats was 128 hours, while the average survival of four controls was 90 hours. There were not enough experiments to justify any conclusions, but the results were so striking in view of the poor condition of the cholesterol-fed rats that it is difficult to believe that they were due entirely to chance. At least they were a stimulus to further studies.

In series II half of the rats had been fed synthetic diets A and B; the other half had received natural-food diets C and D (table 1). In three pairs on the synthetic diets the cholesterol-fed rat survived its control, in seven pairs the contrary was the case, while in one pair both rats survived the same time. In six pairs on the natural-food diets both rats recovered from the infection, in two pairs the cholesterol-fed rat survived its control, and in three pairs the reverse was true. Obviously, cholesterol showed no protective action in this series.

In all cases a pair of rats on the synthetic diet and a pair on the natural-food diet were injected at the same time with the same amounts of the same culture. In seven of the eleven groups all four rats were litter mates. In six of these groups the rats fed natural foods recovered from the infection, while most of the rats fed synthetic diets succumbed. In the remaining five groups, in all of which the rats were somewhat older, no difference in the resistance of rats on synthetic and natural-food diets was evident.

In series III the rats were started at varying times over a period of several months so that animals of different ages might be injected at the same time with the same culture. A few pairs were utilized in determining the proper dose, which was calculated from bacterial counts of the cultures used in

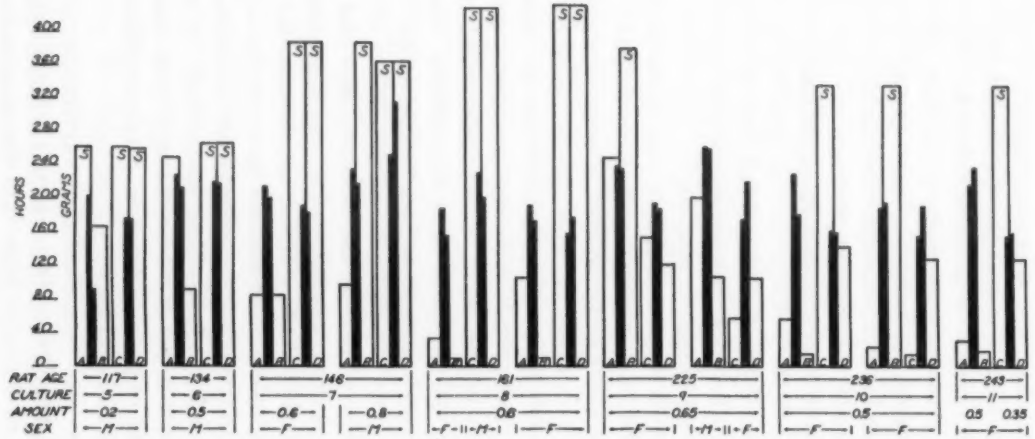

Fig. 1 Survival of rats of series II after inoculation with Salmonella dansyz. Dark bars indicate weight when inoculated. Open bars indicate period of survival in hours. 'S' indicates that rat recovered and was sacrificed. 'A' rats received a synthetic diet without cholesterol. 'B' rats received the same synthetic diet with cholesterol. 'C' rats received a natural-food diet without cholesterol. 'D' rats received the same natural-food diet with cholesterol.

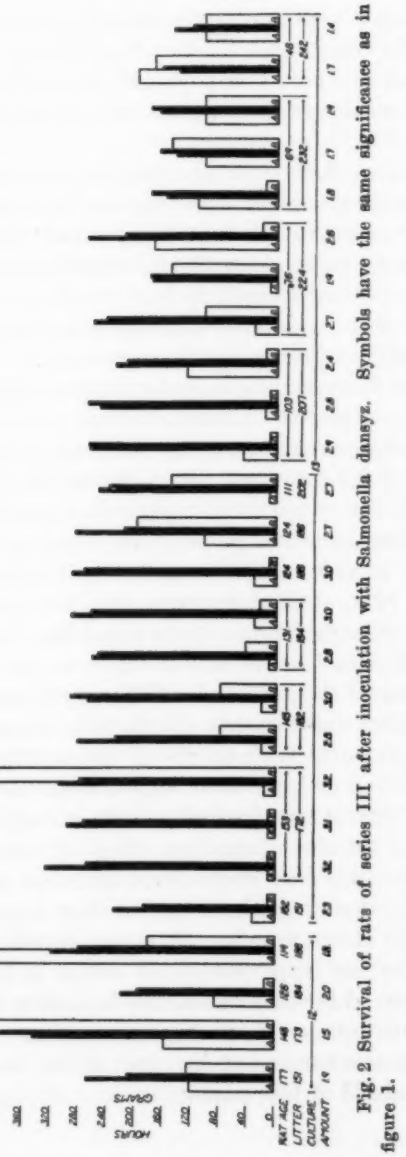

Fig. 2 Survival of rats of series III after inoculation with Salmonella daneyz. Symbols have the same significance as in figure 1.

the preliminary tests and in the inoculation of the main group of rats. The dose was made proportional to the average weight of the two rats of a pair but, as in all of these experiments, both members of a pair received the same amount of culture.

In four pairs there was no difference in time of survival, in eleven pairs the cholesterol-fed rat survived the control, while in eleven pairs the control survived the cholesterol-fed rat. Evidently, as in series II, cholesterol exerted no influence whatsoever. There seemed to be a tendency for the younger rats to show more resistance than the older, but the differences were too small to justify further comment.

Altogether forty-eight pairs of rats were studied in series II and III; in sixteen the cholesterol-fed rat survived its control, in twenty-one the contrary was the case, and in eleven both survived for the same time. Evidently, these results do not support the conclusion under investigation (that cholesterol feeding increases general resistance to infection). We realize that, in view of the large normal variation in the resistance of rats, it is dangerous to draw conclusions from small series of experiments, but we feel that if cholesterol had a general effect in increasing resistance to infection markedly, some evidence of this effect should have appeared in our carefully controlled experiments. It hardly seems likely that in the thirty-two pairs (two-thirds of the total) where the control rat survived as long as or longer than the cholesterol-fed animal, the control rat chanced to have enough excess natural resistance to counterbalance an effect of cholesterol. It is, of course, possible that cholesterol exercises a protective action against other organisms and it may increase resistance to Salmonella dansyz under abnormal conditions, as is suggested by the few experiments of series I, but even if this should be proved beyond doubt, the ingestion of this sterol in excessive amounts as a protective measure by humans is hardly to be recommended in view of the results described in parts I and III of this paper.

III. CHOLESTEROL FEEDING AND INFECTION IN RELATION TO THE CHOLESTEROL OF TISSUES AND BLOOD[19]

Relatively few studies of free and combined cholesterol of tissues have been reported, largely because the only method available until recently (digitonin gravimetric) requires large amounts of tissue and is laborious and time-consuming. The availability of a new, rapid method (Schoenheimer and Sperry, '34) made possible the utilization of the material described in parts I and II in studying the effect of infection and of long-continued cholesterol feeding on tissue cholesterol. Nearly 1000 individual analyses have been made.

Tissues were removed as soon as possible after the death of the animal and a small sample was weighed for moisture determination. In series I and II another sample was weighed, ground with sand, and extracted three times with boiling alcohol followed by three washings with ether. The extracts were filtered into a volumetric flask and made up to volume with alcohol. Free and total cholesterol were determined either by the method of Schoenheimer and Sperry or by a macro-modification thereof. Where the micro procedure was used an aliquot of the alcohol-ether extract was taken to dryness in a small beaker, washed quantitatively with absolute alcohol-acetone (1:1) into a 5-cc. volumetric flask, and made up to volume. From this point on the procedure was the same as described by Schoenheimer and Sperry.

The macro modification was the same in principle as the micro, but the technic differed in several details. It was necessary to concentrate aliquots of the alcohol-ether extract to 2 cc. (free) or 1 cc. (total) in 15-cc. centrifuge tubes. Free cholesterol was precipitated by 1 cc. of 1 per cent digitonin in 40 per cent alcohol. In the determination of total cholesterol alkali was added in the form of sodium wire, the samples were digested 90 minutes at 60°, and acidified with 10 per cent HCl in absolute alcohol. The solution was filtered into another 15-cc. centrifuge tube, using a fritted glass filter with a Pregl siphon, and washing several times with ether,

[19] A preliminary report of this part of the investigation was presented before the American Society of Biological Chemists at New York, March 29, 1934.

which was removed by drawing a stream of air through the tube. The volume was adjusted to 2 cc. with alcohol and digitonin was added as in the determination of free cholesterol. Centrifuging, washing and color development were carried out in essentially the same manner as in the micro procedure. Five-mm. cells (instead of 5-cm.) were used in reading. (We prefer the micro procedure as described by Schoenheimer and Sperry. We were forced to employ the macro method in the early part of this work as at that time 5-cm. micro cells were not available.)

In devising the above procedure a great many control analyses were carried out, similar to those described in connection with the micro method. The accuracy was about the same in both procedures.

Where sufficient material was available most of the determinations were made in duplicate. Duplicates usually agreed within 3 per cent; in a few cases where they differed by more than 5 per cent, the analysis was repeated.

In series III the liver and intestinal tract were removed and the remaining carcass was minced thoroughly by passing several times through a fine grinder. An aliquot was taken for moisture determination and the remainder (or a large aliquot) was extracted completely with hot alcohol in a continuous extractor (Sperry, '26). The alcohol was removed in vacuo, the residue was extracted with ether, the ether solution was dried over sodium sulphate, filtered, and the ether was boiled off. The residue was extracted with petroleum ether, the dried extract was weighed to give total lipids, and an aliquot was dissolved in alcohol-acetone for cholesterol determination as described above. Liver was analyzed by a similar procedure with determination of total petroleum ether-soluble lipids.

Effect of infection on tissue cholesterol. Little is known about the effect of infection on the cholesterol of tissues. Chauffard, Laroche and Grigaut ('12) found low values for the total cholesterol of the adrenals in humans with acute infections. Landau and McNee ('14) confirmed this finding in a few cases and stated that the decrease was largely in

the cholesterol esters. A few investigators (Wacker and Hueck, '13), using histological methods, have found a decrease in the cholesterol esters (doubly-refracting bodies) of the adrenal in acute infection in humans. Baumann and Holly ('25) reported that the total cholesterol of the adrenals was definitely reduced in guinea pigs dying of various infections. The same was true of rabbits, though the results were more variable and increases were observed when the animals were sacrificed in the early stages of the infection.

Save for a few isolated determinations in humans (see Bürger, '28, for a summary), other tissues do not appear to have been studied.

In the present investigation analyses of liver, kidney, lung, adrenal, muscle, skin, and the entire carcass (except for liver and intestinal tract) were made. The data (calculated as percentage cholesterol in dry tissue, except in the case of the adrenal) have been grouped arbitrarily according to the period of survival in order to determine whether any significant changes in tissue cholesterol accompanied the infection (table 6). Average values with probable errors are given for the different groups. Analyses of liver, lung, kidney, and adrenal in rats which had received the natural-food diets C and D are not included, because there were too few data in most of the groups to justify comparisons. The results agree in most instances with those presented in regard to influence of infection. A few rats were sacrificed without inoculation as controls. Rats which recovered from the infection may also be considered as controls, since in most cases results from the 'recovered' and control groups agree closely.

In order to conserve space, the data will not be discussed in detail. The infection appeared to cause significant[11] decreases in 1) the combined cholesterol of the liver[12] and 2) the

[11] The word 'significant' is used to indicate that the difference between averages is more than four times the probable error of the difference.

[12] In the case of cholesterol-fed rats there was also a decrease in the combined cholesterol of the liver, but the differences between groups are not significant, because of a large variation between individuals and a few very high values in the 40- to 120-hour and > 120-hour groups in series I and II. When the largest and best controlled series (III) is considered separately, a highly significant decrease is observed.

TABLE 6

Influence of infection on tissue cholesterol

GROUP	NUMBER OF RATS	DIET[1]	SERIES	CHOLESTEROL		
				Total per cent	Free per cent	Combined per cent
Liver						
Sacrificed[2]	6	A	I, II and III	0.93 ± 0.15	0.65 ± 0.04	0.28 ± 0.11
Survived < 40 hours	16	A	I, II and III	0.85 ± 0.04	0.70 ± 0.02	0.15 ± 0.02
Survived 40 to 120 hours	13	A	I, II and III	0.83 ± 0.03	0.73 ± 0.03	0.10 ± 0.01
Survived > 120 hours	12	A	I, II and III	0.79 ± 0.02	0.74 ± 0.03	0.05 ± 0.01
Recovered[3]	2	A	I, II and III	0.85	0.73	0.12
Sacrificed	5	B	I, II and III	12.59 ± 2.30	0.89 ± 0.05	11.70 ± 2.27
Survived < 40 hours	16	B	I, II and III	10.89 ± 0.75	1.02 ± 0.03	9.87 ± 0.76
Survived 40 to 120 hours	11	B	I, II and III	8.75 ± 0.99	1.14 ± 0.05	7.61 ± 0.95
Survived > 120 hours	10	B	I, II and III	9.20 ± 2.24	1.34 ± 0.15	7.86 ± 2.11
Recovered	8	B	I, II and III	12.72 ± 1.60	0.84 ± 0.03	11.88 ± 1.35
Kidney						
Sacrificed	6	A	I and II	1.71 ± 0.07	1.58 ± 0.06	0.13 ± 0.04
Survived < 40 hours	3	A	I and II	1.80	1.78	0.02
Survived 40 to 120 hours	6	A	I and II	1.60 ± 0.05	1.51 ± 0.04	0.09 ± 0.02
Survived > 120 hours	6	A	I and II	1.68 ± 0.07	1.58 ± 0.06	0.10 ± 0.02
Recovered	1	A	I and II	1.81	1.76	0.05
Sacrificed	5	B	I and II	2.18 ± 0.22	1.81 ± 0.18	0.37 ± 0.08
Survived < 40 hours	5	B	I and II	2.20 ± 0.09	1.90 ± 0.03	0.30 ± 0.06
Survived 40 to 120 hours	5	B	I and II	2.19 ± 0.11	1.96 ± 0.14	0.23 ± 0.02
Survived > 120 hours	3	B	I and II	2.02	1.66	0.36
Recovered	6	B	I and II	2.18 ± 0.08	1.85 ± 0.03	0.33 ± 0.06
Lung						
Sacrificed	6	A	I and II	2.40 ± 0.19	2.17 ± 0.14	0.23 ± 0.03
Survived < 40 hours	3	A	I and II	1.65	1.56	0.09
Survived 40 to 120 hours	6	A	I and II	1.41 ± 0.11	1.23 ± 0.10	0.18 ± 0.02
Survived > 120 hours	7	A	I and II	1.54 ± 0.04	1.38 ± 0.03	0.16 ± 0.01
Recovered	1	A	I and II	2.12	2.00	0.12
Sacrificed	5	B	I and II	2.77 ± 0.12	2.00 ± 0.07	0.77 ± 0.08
Survived < 40 hours	5	B	I and II	2.71 ± 0.11	2.13 ± 0.14	0.58 ± 0.08
Survived 40 to 120 hours	5	B	I and II	2.12 ± 0.11	1.50 ± 0.11	0.62 ± 0.08
Survived > 120 hours	3	B	I and II	3.44	2.23	1.21
Recovered	6	B	I and II	3.52 ± 0.41	2.34 ± 0.13	1.18 ± 0.33
Adrenal[4]						
Sacrificed	6	A	I and II	5.28 ± 0.62	0.46 ± 0.05	4.82 ± 0.58
Survived < 40 hours	3	A	I and II	0.65	0.26	0.39
Survived 40 to 120 hours	6	A	I and II	0.61 ± 0.08	0.21 ± 0.02	0.40 ± 0.07
Survived > 120 hours	6	A	I and II	0.77 ± 0.05	0.25 ± 0.02	0.52 ± 0.05
Recovered	1	A	I and II	2.95	0.39	2.56
Sacrificed	5	B	I and II	9.39 ± 1.14	0.51 ± 0.07	8.88 ± 1.08
Survived < 40 hours	5	B	I and II	5.16 ± 0.49	0.76 ± 0.09	4.40 ± 0.43
Survived 40 to 120 hours	5	B	I and II	2.03 ± 0.45	0.31 ± 0.04	1.72 ± 0.36
Survived > 120 hours	3	B	I and II	3.32	0.81	2.51
Recovered	6	B	I and II	10.46 ± 1.09	0.65 ± 0.06	9.81 ± 1.06
Carcass[5]						
Survived < 40 hours	12	A	III	0.41 ± 0.02	0.27 ± 0.01	0.14 ± 0.01
Survived 40 to 120 hours	7	A	III	0.56 ± 0.03	0.35 ± 0.01	0.21 ± 0.02
Survived > 120 hours	5	A	III	0.55 ± 0.09	0.34 ± 0.05	0.21 ± 0.04
Recovered	1	A	III	0.49	0.32	0.17
Survived < 40 hours	9	B	III	0.51 ± 0.02	0.32 ± 0.01	0.19 ± 0.01
Survived 40 to 120 hours	5	B	III	0.66 ± 0.06	0.42 ± 0.04	0.24 ± 0.02
Survived > 120 hours	7	B	III	0.79 ± 0.05	0.50 ± 0.03	0.29 ± 0.03
Recovered	2	B	III	0.37	0.25	0.12

[1] A was a synthetic, cholesterol-free diet. B was exactly the same, except for the addition of cholesterol.

[2] Rats in the 'sacrificed' groups had not been injected with a bacterial culture.

[3] When sacrificed, rats in the 'recovered' groups were eating well and had recovered completely from the infection as judged by general appearance.

[4] Values for the adrenal are calculated as percentage of fresh tissue.

[5] Liver and intestinal tract were removed. See text.

free cholesterol of the lung in rats which had received no cholesterol in the diet, and 3) in the combined cholesterol of the adrenal both in rats which had and had not been fed cholesterol. Significant increases in both free and combined cholesterol of the carcass accompanied the infection. Muscle and skin were analyzed in rats of series I. There were too few determinations to justify detailed presentation, but it is of interest that the results agree with the findings on the carcass in showing marked increases in cholesterol accompanying the infection. A decrease in the combined cholesterol of the lung and an increase in the free cholesterol of the liver were also noted, but the differences were not significant. There was no change in the kidneys.

TABLE 7

Total lipids in carcass and liver. Series III

GROUP	TOTAL LIPIDS				DIFFERENCE BETWEEN TOTAL LIPIDS AND CHOLESTEROL ESTERS OF LIVER DIET B
	In carcass		In liver		
	Diet A	Diet B	Diet A	Diet B	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Survived < 40 hours	46.6 ± 2.3	50.8 ± 2.3	15.2 ± 0.3	28.9 ± 1.0	13.0 ± 0.3
Survived 40 to 120 hours	35.0 ± 2.1	35.6 ± 2.8	16.8 ± 0.8	23.4 ± 1.1	16.3 ± 0.4
Survived > 120 hours	35.1 ± 3.0	31.0 ± 0.9	14.5 ± 0.6	20.8 ± 1.0	15.3 ± 0.7

We do not feel justified in drawing final conclusions from our data, since there are too few determinations in many of the groups to satisfy strict statistical requirements. A transport of cholesterol from glandular tissue, such as liver and adrenal, probably to the muscles and skin under the influence of the infection is indicated.

Effect of infection on total (petroleum ether-soluble) lipids. Petroleum ether-soluble lipids of the carcass and liver were determined in series III. The results are summarized in table 7. The lipids of the carcass decreased as the infection progressed, but the differences are not statistically significant in the case of rats which had not received cholesterol. It is surprising that the decreases were not larger, in view of the

severity of the infection and the failure, for the most part, of the animals to eat.

There was no appreciable change in liver lipids of rats which had received no cholesterol. In rats which had received cholesterol there was a significant decrease as the infection progressed. The individual percentages of cholesterol esters (calculated as cholesterol oleate from the combined cholesterol values) were subtracted from the corresponding total lipid percentages. The average differences are very nearly the same as the total lipid percentages found in rats which had not received cholesterol. The variation is less than was shown by the total lipid values. Apparently, the increase in liver lipids on the cholesterol diet and the decrease accompanying the infection were entirely in the cholesterol ester fraction.

The influence of cholesterol feeding on the cholesterol of tissues. Several investigators have reported recently that long-continued cholesterol feeding causes large increases in the cholesterol of the liver (p. 131). In agreement with this work, our data show an enormous increase in combined cholesterol of the liver in rats which received cholesterol in the diet (table 6).

Chanutin and Ludewig ('33) found the total lipids of the liver to be increased markedly by cholesterol feeding. Calculation from average percentages shows that the increase was considerably greater than can be accounted for by increased cholesterol esters. Okey ('33) also found a greater increase in the total fatty acids of the liver than the fatty acids combined with cholesterol could explain. In our experiments the increase in liver lipids following cholesterol feeding was very nearly the same as the increase in cholesterol esters (table 7). We have no explanation of this discrepancy. The negligible amount of cholesterol esters in the livers of rats which had not received cholesterol was disregarded in making these calculations.

In this connection it is interesting that cholesterol feeding caused no increase in the lipids of the carcass.

Okey reports no effect of cholesterol feeding on the free cholesterol of the liver, while Chanutin and Ludewig found an increase. Our data on free cholesterol of the liver of rats which had not received cholesterol have been compared with the corresponding values for rats which had received cholesterol (table 8). The results have been tabulated by groups according to the survival of the animals in order to rule out the effect of the infection as far as possible. In all groups the percentages are higher in the case of cholesterol-fed rats than in rats which had not received cholesterol and in most instances the differences are significant.

TABLE 8

Effect of cholesterol feeding on free cholesterol of liver

GROUP	SERIES	FREE CHOLESTEROL		DIFFERENCE	PROBABLE ERROR OF DIFFERENCE
		Without cholesterol in diet	With cholesterol in diet		
		per cent	*per cent*		
Sacrificed	All	0.65 ± 0.04	0.89 ± 0.05	0.24	0.06
Survived < 40 hours	All	0.70 ± 0.02	1.02 ± 0.03	0.32	0.07
Survived 40 to 120 hours	All	0.73 ± 0.03	1.14 ± 0.05	0.41	0.06
Survived > 120 hours	All	0.74 ± 0.03	1.34 ± 0.15	0.60	0.15
Survived < 40 hours	III	0.71 ± 0.02	0.96 ± 0.02	0.25	0.03
Survived 40 to 120 hours	III	0.74 ± 0.04	1.02 ± 0.05	0.28	0.06
Survived > 120 hours	III	0.73 ± 0.06	1.03 ± 0.06	0.30	0.08

Chanutin and Ludewig are the only authors who have studied the effect of cholesterol feeding on the cholesterol of other tissues than liver in the rat. They conclude that there were no changes in the tissues which they studied (kidney, heart and brain). We have analyzed our data on other tissues in the same manner employed for free cholesterol of the liver (table 9). Cholesterol-fed rats showed a higher average cholesterol content than controls in every group compared, except one. Although in the majority of cases the differences are not statistically significant, due to a considerable individual variation, we feel that the consistency of the results and the relatively large percentage increases in most cases indicate a

tendency for cholesterol to deposit in tissues generally during long-continued cholesterol feeding. These deposits are, however, insignificant when compared with the enormous in-

TABLE 9

Effect of cholesterol feeding on the cholesterol content of various tissues

GROUP	TISSUE	CHOLESTEROL FRACTION	CHOLESTEROL		INCREASE ON CHOLESTEROL DIET
			Without cholesterol in diet	With cholesterol in diet	
			per cent	*per cent*	*per cent*
Sacrificed	Kidney	Total	1.71 ± 0.07	2.18 ± 0.22	27.5
Survived 40 to 120 hours	Kidney	Total	1.60 ± 0.05	2.19 ± 0.11	36.9
Sacrificed	Kidney	Free	1.58 ± 0.06	1.81 ± 0.18	14.6
Survived 40 to 120 hours	Kidney	Free	1.51 ± 0.04	1.96 ± 0.14	29.8
Sacrificed	Kidney	Combined	0.13 ± 0.04	0.37 ± 0.08	184.6
Survived 40 to 120 hours	Kidney	Combined	0.09 ± 0.02	0.23 ± 0.02	155.6
Sacrificed	Lung	Total	2.40 ± 0.19	2.77 ± 0.12	15.4
Survived 40 to 120 hours	Lung	Total	1.41 ± 0.11	2.12 ± 0.11	50.4
Sacrificed	Lung	Free	2.17 ± 0.14	2.00 ± 0.07	— 7.8
Survived 40 to 120 hours	Lung	Free	1.23 ± 0.10	1.50 ± 0.11	22.0
Sacrificed	Lung	Combined	0.23 ± 0.03	0.77 ± 0.08	234.8
Survived 40 to 120 hours	Lung	Combined	0.18 ± 0.02	0.62 ± 0.08	244.4
Sacrificed	Adrenal	Total	5.28 ± 0.62	9.39 ± 1.14	77.8
Survived 40 to 120 hours	Adrenal	Total	0.61 ± 0.08	2.03 ± 0.45	232.8
Sacrificed	Adrenal	Free	0.46 ± 0.05	0.51 ± 0.07	10.9
Survived 40 to 120 hours	Adrenal	Free	0.21 ± 0.02	0.31 ± 0.04	47.6
Sacrificed	Adrenal	Combined	4.82 ± 0.58	8.88 ± 1.08	84.2
Survived 40 to 120 hours	Adrenal	Combined	0.40 ± 0.07	1.72 ± 0.36	330.0
Survived < 40 hours	Carcass	Total	0.41 ± 0.02	0.51 ± 0.02	24.4
Survived 40 to 120 hours	Carcass	Total	0.56 ± 0.03	0.66 ± 0.06	17.9
Survived > 120 hours	Carcass	Total	0.55 ± 0.09	0.79 ± 0.05	43.6
Survived < 40 hours	Carcass	Free	0.27 ± 0.01	0.32 ± 0.01	18.5
Survived 40 to 120 hours	Carcass	Free	0.35 ± 0.01	0.42 ± 0.04	20.0
Survived > 120 hours	Carcass	Free	0.34 ± 0.05	0.50 ± 0.03	47.1
Survived < 40 hours	Carcass	Combined	0.14 ± 0.01	0.19 ± 0.01	35.7
Survived 40 to 120 hours	Carcass	Combined	0.21 ± 0.02	0.24 ± 0.02	14.3
Survived > 120 hours	Carcass	Combined	0.21 ± 0.04	0.29 ± 0.03	38.1

creases in combined cholesterol of the liver. In several of our cholesterol-fed rats over half of the total body (except intestinal tract) cholesterol was present in the liver.

The cholesterol of the blood serum was determined in rats which were sacrificed without infection and which recovered

from the infection (table 10). Cholesterol feeding caused large increases in all cholesterol fractions. The effect was much more marked with synthetic than with natural-food diets (p. 136).

It is evident that omnivorous animals, such as the rat, do not differ from herbivorous animals in the ability to absorb cholesterol and deposit it in tissues. They appear to differ markedly in regard to cholesterol deposits in the arteries. Aortas from most of the cholesterol-fed rats were examined by Dr. Beryl Paige, of the Department of Pathology, Babies'

TABLE 10

Cholesterol in serum

GROUP	NUMBER OF RATS	DIET	CHOLESTEROL		
			Total mg./100 cc.	Free mg./100 cc.	Combined mg./100 cc.
Sacrificed	5	A	59.9 ± 2.6	18.4 ± 1.9	41.5 ± 1.0
Recovered	2	A	59.1	17.3	41.8
Sacrificed	5	B	231.4 ± 29.1	48.1 ± 8.1	183.3 ± 22.3
Recovered	8	B	211.7 ± 14.7	56.1 ± 4.4	155.6 ± 10.9
Sacrificed	2	C	59.6	18.1	41.5
Recovered	8	C	51.2 ± 2.8	12.4 ± 1.0	38.8 ± 2.2
Sacrificed	2	D	95.0	20.8	74.2
Recovered	6	D	108.1 ± 11.0	25.6 ± 2.6	82.5 ± 8.6

Hospital. No arteriosclerotic lesions were discovered. Under the same dietary conditions, marked lesions, involving large cholesterol deposits, would have been expected in rabbits.

SUMMARY

Long-continued ingestion of cholesterol at a high level has been studied in relation to growth, efficiency of food utilization, resistance to infection, and deposition of cholesterol in tissues.

Cholesterol-fed rats grew less well, ate less food, and utilized their food less efficiently than controls receiving the same synthetic diet without cholesterol. These effects are most probably related to the large deposits of cholesterol

esters found in the livers of the cholesterol-fed rats, since they were not observed in a group of rats (receiving a natural-food diet plus cholesterol) in which the deposits of cholesterol were relatively small.

Except in a small series complicated by a probable vitamin G deficiency rats fed diets high in cholesterol for long periods of time showed no more resistance to the paratyphoid organism Salmonella dansyz than controls fed exactly the same diet without cholesterol.

Free, total and combined cholesterol have been determined in various tissues of the rats used in the foregoing studies. Decreases in the cholesterol of liver, adrenal and lung and increases in the cholesterol of the entire carcass (except for liver and intestinal tract) accompanied the paratyphoid infection. No changes were observed in kidney cholesterol.

Increases in all cholesterol fractions of all tissues studied followed cholesterol feeding, but the increase was large only in the combined cholesterol of the liver.

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THE EFFECT OF DIET ON LIVER CHOLESTEROL IN CHICKENS [1]

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Little attention has been paid to the cholesterol of bird tissues or the effect of diet thereon. Several investigators (Yamagouchi, '25; Anitschkow, '25; Uchiyama, '30) have reported deposits of cholesterol esters (anisotropic droplets) in the livers of chickens and pigeons following the feeding of cholesterol. From these results it would appear that cholesterol is absorbed and deposited by birds as it is by mammals; but the only chemical analyses, of which we are aware, are those of Hotta ('24), who determined the total cholesterol of a number of tissues in the pigeon following cholesterol feeding. Marked increases in the cholesterol of liver, muscle, spleen, gonads and blood, and small increases in adrenal, pancreas, and brain cholesterol followed the ingestion of large quantities (0.25 to 0.50 gm. per pigeon per day) of cholesterol over a period of about 1 month. Unfortunately, from the standpoint of the problem at hand, these experiments were complicated by the presence of beri-beri. The diet consisted only of polished rice and cholesterol. The experiments were suggested by the finding of Lawaczeck ('23) and Hotta ('23) (later confirmed by Messerle, '25) that the cholesterol of various tissues (liver, muscle, spleen, pancreas, brain and blood) is increased in beri-beri. The increases found by Hotta after cholesterol feeding were little, if any,

[1] This investigation was supported in part by the Josiah Macy, Jr., Foundation.

greater in most tissues than the increases caused by beri-beri per se.

We have been fortunate in obtaining the livers[2] from a number of chickens which had been fed diets varying widely in cholesterol content under carefully controlled conditions in the course of an investigation of the nutritional encephalomalacia described by Pappenheimer and Goettsch ('31, '33, '34). Free, total, and combined cholesterol have been determined in these livers.

EXPERIMENTAL

The extraction of the livers and the determination of cholesterol were carried out in the manner described in the preceding paper (Sperry and Stoyanoff, '35). The micro method was used exclusively for the cholesterol analyses.

DISCUSSION

The results, expressed as percentage of dry weight of liver, are presented in table 1. On diet 634, which was low in cholesterol, the values are quite uniform. The total cholesterol percentages are about the same as those found in rats which received a synthetic diet without cholesterol (diet A) (table 6 of the preceding paper), but the free cholesterol is slightly higher and the combined cholesterol is lower.

Diet 108, although low in cholesterol, probably contained more of this sterol than diet 634, since it was comprised largely of substances (lard, casein and skimmed milk powder) containing small percentages of cholesterol. A definite tendency to deposit cholesterol esters in the liver was exhibited by chicks on this diet. Most of the values are higher than were found with diet 634. (Note especially chick 1409.)

The commercial lecithin, which was fed along with diet 108 in the third series, may have contained small amounts of cholesterol. Most of the chicks showed no greater deposits than the controls on diet 108, but both free and combined

[2] We are greatly indebted to Dr. Alwin M. Pappenheimer and Dr. Marianne Goettsch for this material.

TABLE 1

Cholesterol in chicken livers on different diets

CHICK NO.	DAYS ON DIET	CHOLESTEROL		
		Total per cent	Free per cent	Combined per cent
Natural-food diet 634[1]				
1488	33	0.833	0.776	0.057
1362	37	0.908	0.855	0.053
1475	37	0.928	0.844	0.084
1479	37	1.021	0.942	0.079
1485	44	0.783	0.735	0.048
1477	53	0.718	0.676	0.042
Diet 108[1]				
1408	26	1.071	0.873	0.198
1415	28	0.879	0.786	0.093
1413	49	0.996	0.835	0.161
1404	50	1.011	0.829	0.182
1402	56	1.000	0.942	0.058
1407	56	0.846	0.714	0.132
1409	56	1.471	1.143	0.328
1414	59	0.992	0.802	0.190
1418	59	0.896	0.826	0.070
Diet 108 + 0.1 gm. lecithin[2] per chick per day				
1436	24	1.209	0.996	0.213
1439	25	0.972	0.851	0.121
1448	30	1.648	1.535	0.113
1441	34	1.020	1.044	....
1454	41	0.955	0.835	0.120
1452	42	1.051	0.923	0.128
1456	42	0.858	0.757	0.101
1437	47	2.898	1.818	1.080
1446	49	0.865	0.844	0.021
1453	49	1.702	1.256	0.446
Diet 108 + 1 cc. egg yolk per chick per day				
1432	40	4.177	2.578	1.599
1421	47	0.811	0.708	0.103
1426	49	1.113	0.911	0.202
1420	50	0.931	0.795	0.136
1423	50	1.107	0.847	0.260
1424	50	0.955	0.852	0.103
1428	50	0.909	0.810	0.099
1430	50	0.982	0.804	0.178
1433	50	0.704	0.644	0.060
Diet 108 + 5 gm. calf brain per chick per day				
1466 1473 1460	24	6.891	1.874	5.017
1469	42	1.691	1.086	0.605
1463	44	3.024	1.888	1.136
1457	47	1.560	1.226	0.334
1458	59	1.264	1.000	0.264
1459	59	1.599	1.353	0.246
1461	59	1.613	1.148	0.465
1462	59	4.116	1.660	2.456
1464	59	1.949	1.277	0.672
1465	59	1.784	1.251	0.533
1468	59	1.130	0.891	0.239
1471	59	3.098	1.340	1.758
1472	59	2.817	1.599	1.218
1467	61	2.201	1.664	0.537
1470	61	1.694	1.399	0.295

[1] See Pappenheimer and Goettsch ('33) for the composition of diets 634 and 108.

[2] Commercial lecithin was used.

cholesterol were exceptionally high in the case of chick 1437, while the free cholesterol was elevated in the case of chick 1448.

The 1 cc. of egg yolk fed with diet 108 in the fourth series may be assumed to have contained approximately 15 mg. of cholesterol since most published analyses of egg yolk show a cholesterol content of about 1.5 per cent. This amount showed no effect, except in chick 1432, where very high values for both free and combined cholesterol were obtained.

There is wide variation in the published analyses of cholesterol in brain. The average is about 2 per cent, which would be equivalent to an extra intake of 100 mg. of cholesterol per chick per day in the fifth series where 5 gm. of brain were fed along with diet 108. There was an unmistakable increase in all liver cholesterol fractions in most of the chicks on this diet, but with a few exceptions the deposits of combined cholesterol were relatively small. The increase was as marked in the free as in the combined cholesterol, in contrast to the finding with rats where the increases in free cholesterol after cholesterol feeding were relatively small (table 8 of the preceding paper). The largest deposits were found in the three chicks (1466, 1473 and 1460, pooled for analysis) which had been on the diet only 24 days as compared with 42 days or more in the case of the remaining chicks. The livers of these chicks were loaded with anisotropic bodies. In this connection it is interesting that Schönheimer and Yuasa ('29) found that the blood cholesterol reached a maximum after about 40 days of cholesterol feeding and then fell off rapidly in rabbits. Unfortunately, no liver analyses were done.

SUMMARY

Total, free and combined cholesterol have been determined in the livers of chicks which had been fed diets of varying cholesterol content.

The results show that chickens behave like mammals in absorbing cholesterol and depositing it in the liver, but they differ from rats in depositing proportionately more free cholesterol.

Occasional chicks showed exceptionally high cholesterol values on diets which were low in cholesterol, indicating a marked individual variation in ability to assimilate this sterol.

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VITAMINS A, D AND B IN OYSTERS—EFFECT OF COOKING UPON VITAMINS A AND B[1]

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THREE FIGURES

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Oysters are one of the few animal foods in which the entire animal is eaten. It has been repeatedly demonstrated (McCollum, '31) that the visceral parts of animals usually contain larger vitamin reserves than the muscle meat. Since no part of an oyster, except the shell, is discarded in preparing it for food, the active tissue as well as the storage tissue is consumed. The food of the oyster consists of marine plankton of which diatoms are an important part (Savage, '25). To these minute forms has been attributed the original synthesis of the fat-soluble vitamins which certain fish, notably the cod and the halibut, are able to concentrate in their livers (Coward and Drummond, '21).

The American oyster, Ostrea virginica, inhabits the tidal waters of bays and estuaries of the United States from Massachusetts to the Gulf of Mexico. With very few exceptions, all the marketable oysters from the Atlantic coast waters are of the same species, but, pursuant to varying local customs within the industry, oysters north of the Chesapeake are maintained on the beds for from 3 to 5 years before marketing, whereas further south the stock is harvested at from 1 to 2

[1] This investigation was conducted in part under a grant from Dr. H. D. Pease, director of the Research Committee of the Oyster Growers and Dealers Association of North America.

years of age. The English species, O. edulis, and the American Pacific coast oyster, O. lurida, are quite similar. The only other oyster of commercial significance on this continent is the Japanese oyster, O. gigas, of the west coast.

Each species, and, indeed, oysters of the same species from various sections, has its individual market where its particular qualities of size, shape and flavor are in demand.

Jones, Murphy and Nelson ('28) made a survey of the vitamin content of shellfish in 1928 and their work indicated that the southern oysters with which they worked contained good stores of vitamins A, B and D. Randoin ('23) demonstrated vitamin C in oysters from French tidal waters in 1923.

Methods of vitamin assay have been further standardized since Jones, Murphy and Nelson made their studies, and, in addition, vitamin B has been separated into more than one component. It was, therefore, thought of interest to review the problem of the vitamin content of oysters, using O. virginica from northern waters for the study. Since a large percentage of this food is cooked before it is eaten, the effect of this treatment on the heat-sensitive vitamins was included in the present investigation.

METHODS

Oysters.[2] A haul of oysters large enough for all the present studies was taken from Great South Bay, Long Island, New York, in October of 1932, immediately shucked, washed in sea water, and 'quick-frozen' in half-pound packages by the 'Birdseye process' at a temperature of —33°C. The frozen oysters were kept at a temperature of —10°C. until used. When biological material is frozen at a temperature as low as —33°C., it passes through the critical range so rapidly that ice crystals do not form and hence damage to cell structures is almost completely avoided.

For the vitamin assays, graded doses of raw and cooked oysters were fed to rats. A mass of raw, frozen oysters was

[2] We are indebted to Mr. W. H. Raye, president of the Bluepoints Company, for this supply of oysters.

ground up in a mortar, weighed out, and fed immediately. Cooked oysters were prepared as follows: Six whole oysters were removed from the package, placed in a beaker with no additional water, and kept on a steam bath for 10 minutes at which time the edges of the oysters' mantles curled. They were then removed, immediately ground in a mortar, weighed and fed. All moisture which exuded from the oysters during cooking was thoroughly mixed with the meats during grinding. The rats consumed the oysters very readily.

Chemical analyses were made of the frozen oysters.

TABLE 1

Chemical composition of raw oysters

	Per cent
Moisture	77.35
Total solids	22.65
Total nitrogen	1.59
Protein (N × 6.25)	9.95
Fat	1.45
Ash	1.69
Salt (NaCl)	0.69
Glycogen	6.14
Calcium as CaO	0.10
Magnesium as MgO	0.09
Sulfur (S)	0.09
Phosphorus as P_2O_5	0.30
Lead	Absent
Mercury	Absent
Arsenic	1 part per million
Copper	67 parts per million
Iron	33 parts per million
Aluminum	134 parts per million

The moisture content of the oysters after cooking was 75.00 per cent.

Rat assays of vitamin A, B and D

The rats were of Wistar Institute strain. Since growth is used quantitatively as a criterion of the vitamin content for vitamins A and B, the treatment of the rat colony and of the young rats previous to the experiment is important and

is therefore given. The breeding colony was kept on the following diet:

Colony diet

	Per cent
Whole ground wheat	35
Whole ground corn	32
Whole milk powder	21
Alfalfa leaf meal	2
Old process linseed oil meal	7
Liver, vacuum dried	0.5
Yeast	2.0
$CaCO_3$	0.5
NaCl	0.5

When litters were 10 days old, the mother was transferred to

Vitamin restricted diet

	Per cent
Whole ground wheat	33
Whole ground corn	34
Skimmed milk	21
Old process linseed oil meal	7
Yeast	4
$CaCO_3$	0.5
NaCl	0.5

The young rats were weaned at 21 days of age, at which time the mother was put back on the Colony diet and the young were continued on the vitamin restricted diet until they were 28 days of age. Only young rats weighing between 40 and 50 gm. were used for the experiment.

When ready for the experiment, the young rats were transferred to the vitamin-deficient ration. Rats to be used for the vitamin A and B experiments were allowed to remain on the deficient ration until growth began to decline. At that time, they were divided into groups of eight to ten rats, comparable with respect to sex and litter, and fed graded doses of raw and cooked oysters. One group was always kept as a negative control, and one group was given a known amount of standard source of the vitamin in question.

Our vitamin-deficient diets were as follows:

Vitamin A deficient diet	*Per cent*	*Vitamin B(B_1) deficient diet*	*Per cent*
Casein (vitamin A and B free)	18	Casein (vitamin A and B free)	18
Starch	60	Starch	53
Yeast	8	Autoclaved yeast	15
Salt mixture (Osborne and Mendel)	4	Salt mixture (Osborne and Mendel)	4
Lard	8	Lard	8
Viosterol (1: 1000)	2	Cod liver oil	2

The vitamin A method used is that approved by the U.S.P. X Revision Committee, and the vitamin B method that of Chase and Sherman ('31). The vitamin D method used is that of the U.S.P. X Revision Committee in which the new

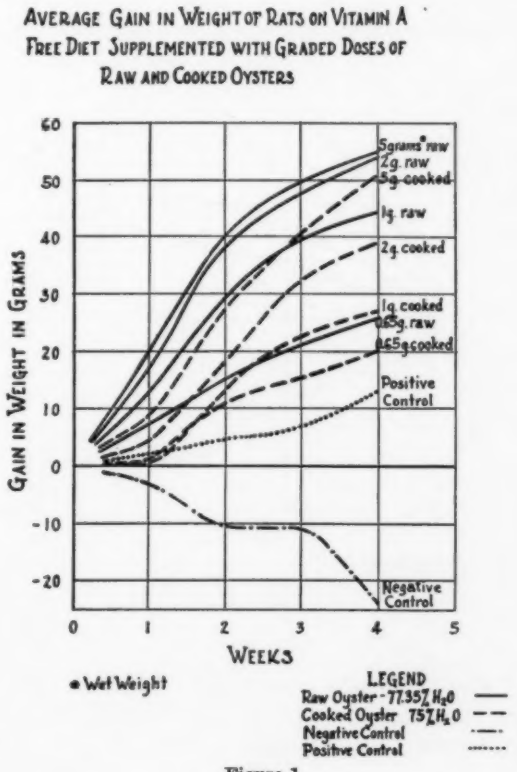

Figure 1

international irradiated ergosterol is used as standard. Vitamin D potency is expressed in vitamin D units U.S.P. (1934 revision) in which 1 unit is the antirachitic potency of 1 mg. of the standard solution. One of these units is approximately equivalent to 0.37 Steenbock units.

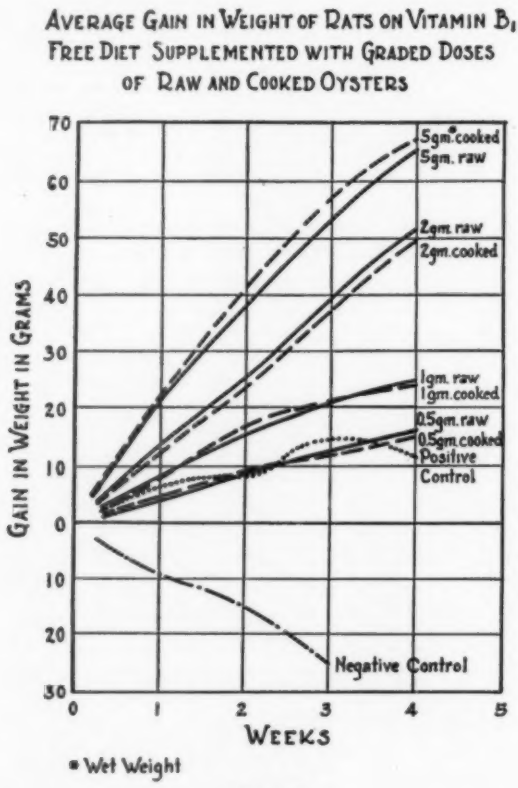

Figure 2

RESULTS

Figure 1 shows the data concerning vitamin A. The smallest dose of raw oyster given (0.65 gm.) permitted an average gain of 26.2 gm. in the 4-week period, a little more than twice the

standard gain allowed for 1 unit of the vitamin. On this basis 1 gm. of oyster contained approximately 3 units of vitamin A. At all levels of feeding the cooked oyster was slightly lower in vitamin A content than the raw; 1 gm. of cooked oyster contained approximately 2.3 units of vitamin A.

Values for vitamin B(B_1) are given in figure 2. The lowest level of feeding in this experiment permitted an average gain of 16 gm. in the 4-week period, only very slightly more growth

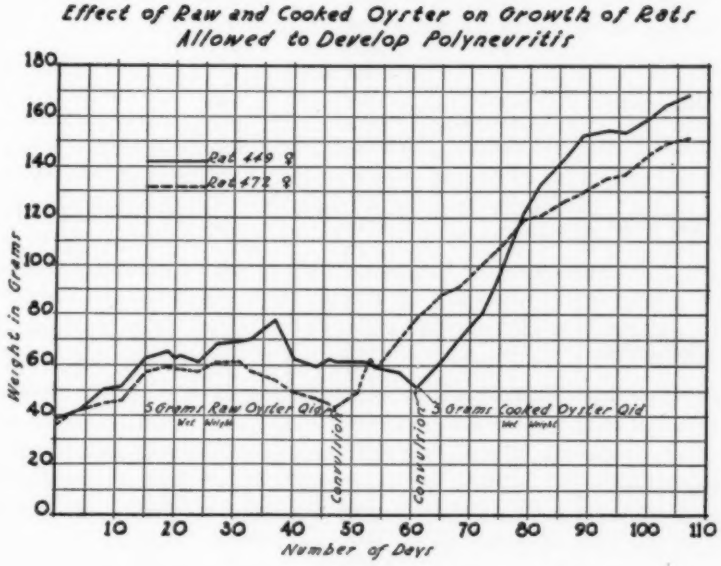

Figure 3

than that allowed for 1 Sherman unit of the vitamin. It can be said that 1 gm. of oyster contains approximately 2.0 units of vitamin B(B_1). The cooking had practically no effect upon the vitamin B(B_1) content.

Two rats were permitted to develop severe beri-beri. During the height of the disease, when the animals were suffering from severe neuro-muscular disturbances, they were given 5 gm. of raw oyster and 5 gm. of cooked oyster, respectively. The results are given on figure 3 and show that both supple-

ments caused an immediate gain in weight which was accompanied in each case by a rapid disappearance of the symptoms and a return to normal.

TABLE 2

Line tests on radii of rats fed raw oyster

SUPPLEMENT	DOSE	RAT NO.	LINE TEST	AVERAGE LINE TEST
	In gm.			
Raw oyster	5	1056	0	
	5	1063	0	0
	5	1070	0	
	5	1057	0	
	5	1064	0	
Raw oyster	10	1112	1+	
	10	1094	1+	1
	10	1135	1+	
	10	1120	1+	
	10	1055	1+	
Raw oyster	15	1115	2+	
	15	1095	2+	2.5
	15	1100	3+	
	15	1123	3+	
	15	1097	3+	
Raw oyster	20	1114	4+	
	20	1096	3+	3.8
	20	1116	4+	
	20	1111	4+	
	20	1099	4+	
	In cc.			
Olive oil	1	1121	0	
	1	1136	0	0
	1	1139	0	
	1	1091	0	
	1	1098	0	
Standard	0.02	1080	4+	
irradiated	0.02	1051	4+	4.0
ergosterol	0.02	1072	4+	
1: 10	0.02	1058	4+	
	0.02	1073	4+	

Table 2 shows the data on the line tests for vitamin D. It was necessary to feed doses of 20 gm. in order to obtain healing equal to that of the one unit of irradiated ergosterol. This is a large amount of supplement to feed a rat and

hence the accuracy of the test was not great. However, the test showed that these oysters contained approximately 1 vitamin D unit (U.S.P.) per 20 gm. of oyster or 0.05 unit per gram.

TABLE 3

Units of vitamins A and D in whole oyster and in oyster oil

	UNITS PER GRAM OF TOTAL OYSTER	UNITS PER GRAM OF OYSTER OIL
Vitamin A	3.0	208.0
Vitamin D	0.05	3.4

DISCUSSION

Whole oyster was found to contain approximately 3 U.S.P. units of vitamin A per gram and 0.05 U.S.P. unit of vitamin D. If all the fat-soluble vitamins are assumed to be in the fat of the mollusk, oyster oil is a rich source of vitamin A, though a poor source of vitamin D. The oysters used in these experiments contained 1.45 per cent fat, or 1 unit of vitamin A per 0.0048 gm. of fat, or 208 units of vitamin A per gram of oyster oil, and 1 unit of vitamin D per 0.29 gm. of fat, or 3.4 units of vitamin D per gram of oyster oil. The oyster seems able to concentrate vitamin A to nearly the same degree as the cod, but it is unable to store comparable quantities of vitamin D. Possibly the shell, the exo-skeletal structure of the mollusk, is laid down through quite a different mechanism from that of the vertebrate fishes, and it is conceivable that vitamin D plays either no role, or at least a different one from that which it plays in animals with an internal skeletal system.

The oysters used for these experiments were removed from the water in October, which is not a time when the oyster is actively depositing shell. Since a marked seasonal variation exists in the calcium metabolism of the oyster, it is possible that there might likewise be an annual cycle in vitamin D content. Likewise, the relatively large amount of vitamin A may have some relation to the approaching period of hibernation.

In stating units of vitamin D per gram of food substance, considerable caution must be exercised. The rat responds to the antirachitic action of oysters as compared with standard irradiated ergosterol in such a way that it takes 20 gm. of oysters to equal 0.02 cc. of 1:10 dilution of standard irradiated ergosterol. We are not, however, justified in concluding that a child would require the same relative number of oyster units of vitamin D as a rat. Children and rats apparently respond very differently to vitamin D from varying sources (Hess and Lewis, '33), and, therefore, we can draw no conclusion concerning the antirachitic potency of oysters for children.

The oysters suffered very little loss in vitamin potency during cooking. This was partly due, no doubt, to the fact that the oysters were cooked whole and ground for feeding after cooking. The vitamins are probably present largely in the visceral parts of the oyster and are therefore protected from the surface oxidation which takes place during exposure to cooking temperatures.

It is difficult to make exact comparison of these studies with those of Jones, Murphy and Nelson ('28) on the same subject, because the technic of vitamin assays has been further standardized since their studies were made. They stated that 2 gm. of raw oyster (0.32 gm. on a dry basis) furnished sufficient vitamin A to cure rats of xerophthalmia. These rats gained approximately 35 gm. This is about three times the gain allowed for 1 unit of vitamin A, which signified that the oysters used by the Washington workers contained approximately 1 unit of vitamin A per 1.5 gm. wet weight of oyster. This is approximately half the potency of the oyster used in the present studies. The difference may be due to age of the oyster, the type of food, and the season at which it was removed from the water. (The material used by Jones, Murphy and Nelson was Chesapeake Bay oysters obtained during the winter from the Washington market.)

The oysters used by Jones, Murphy and Nelson contained almost twice as much vitamin D as those described in these

studies. This difference may have been due to the same factors as those which caused the differences in vitamin A potency.

SUMMARY

The vitamin A content of oysters taken from the beds in October was found to be approximately 3 U.S.P. units per gram. Assuming that all of the vitamin A is in the fat of the oyster, oyster oil contained 208 units per gram. There was a slight destruction of vitamin A during cooking.

The vitamin D content of oysters harvested in the fall was found to be approximately 0.05 U.S.P. unit per gram. Assuming that all of this vitamin is also in the fat of the oyster, the oyster oil contained 3.4 units per gram. The discrepancy between rat units of vitamin D and antirachitic potency for children is emphasized.

The vitamin B(B_1) content of oysters was found to be approximately 1.5 Sherman units per gram. There was no appreciable destruction during cooking.

Oysters were found to be an excellent food source of vitamin B(B_1), a relatively good one of vitamin A, and a very modest source of vitamin D.

Whether the vitamin content of oysters varies at different times in the year remains to be determined.

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MINERAL EXCHANGES OF MAN

IV. VARIATIONS IN THE MINERAL CONTENT OF DIETS WITH A CONSTANT RAW WEIGHT FORMULA [1]

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The present communication is intended to serve as a supplement to the first paper in this series (Bassett, Elden and McCann, '31). It deals specifically with the application of a method for approximate quantitative determination of the amount of mineral elements ingested by human subjects during the course of protracted balance experiments. A brief review of the usual methods employed in the estimation of mineral intake is included, since it is pertinent to the present discussion.

1. Estimation of mineral content of food by use of tables of averages

The amount of each of the elements in the diet may be calculated from the raw weights of the different foods ingested by use of average values for such elements as found in the literature. Tables showing these average values have been compiled by Sherman ('26), Rose ('31) and others. The method serves a useful purpose when it is unnecessary or impossible to obtain a more precise estimate of the mineral content of the food ingested.

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2. Estimation of mineral content by analysis of food

a. Actual analyses of the various food stuffs to be used in the diet may be made by the investigator and the values thus obtained employed in calculating the intake. Such a method is capable of giving excellent results when the total number of items on the menu is relatively small and when sufficient quantities of the raw foods may be purchased in advance to insure an adequate supply for the duration of the experiment. It is obviously important that the food be of as uniform a composition as possible and that the source of the individual items be constant. The wide variations in the mineral content of grain grown under different conditions of soil and climate (Greaves and Hirst, '29) serve to emphasize this point. In general, the method would seem more suitable for metabolism experiments on animals or small children where a monotonous diet may be comparatively well tolerated.

b. One or more sample diets duplicating in all respects the diet or diets to be ingested by the subject of the experiment in a metabolic period are prepared, the several items mixed, dried and pulverized. Samples from the composite mixture are then weighed out, ashed and analyzed. The total of each of the mineral constituents of a day's food are computed from these analyses. Complete duplicate analyses of the food ingested in each subsequent metabolic period are not undertaken, but the formulae of the diets to be used are made, in so far as possible, both qualitatively and quantitatively identical with those which have been analyzed. It, therefore, appears logical to employ the values which were derived from the original analyses in making calculations of mineral intake in metabolic periods which follow. This method has been used by Salter, Fulton and Angier ('31) in the calculation of the acid-base content of diets and has also been employed in some of our metabolic work (Bassett, Elden and McCann, '31). It has become increasingly apparent, however, that even when the original menu is accurately duplicated in preparing successively the same mixtures of foods for analysis, the

analyses may reveal considerable period by period variability in the mineral content of the diet.

c. The third method in general use is laborious, but seems to insure greater accuracy. An aliquot portion of each item of food is weighed or measured at the time the diet is prepared and the aliquots are saved for analysis. The samples saved may conveniently be pooled for a day or for several days when the periods are longer. The intake is computed from analysis of the pooled samples which are subjected to the same analytical methods as are used in obtaining the mineral content of the excreta. Technical details of a modification of this procedure are described in subsequent paragraphs.

EXPERIMENTAL

Composition of diets and length of periods

Two diets were chosen for their high and low calcium content, respectively. Tables 1 and 2 give the daily menus of these diets and the total quantity of each item of food given during a 6-day period. Collection periods were fixed at 6 days, since a period of this length has been used most frequently in our metabolism experiments. The high calcium diet (table 1) was sampled for eleven periods (66 days) and the low calcium diet (table 2) for ten periods (60 days).

Method of sampling

A sufficient quantity of raw food materials was assembled to meet the menu requirement for the day, plus an amount which permitted sampling. As each item was weighed or measured for the patient, an aliquot part equal to one-quarter the amount of the serving was saved for analysis. These aliquots were chosen so that they represented as nearly as possible the actual food received by the patient. Samples were transferred from the receptacle in which the measurement was made to a large cylindrical Pyrex glass collection jar. Solid foods were minced prior to transfer. The jar was kept covered and in a refrigerator when not in use. When

meat was served it was ground, mixed, and portions weighed from the ground mixture. The aliquot saved for analysis was cooked in a manner identical to that of the patient's

TABLE 1

High calcium diets. Raw food constituents (edible portions)

FOOD	RAW MEASURE—GRAM OR CUBIC CENTIMETER						TOTALS FOR 6 DAYS
	Day of period						
	1st	2nd	3rd	4th	5th	6th	
Milk	580	750	620	780	720	520	3970
Cheese	30		30			30	90
Egg	220	210	130	190	190	200	1140
Bread	40	40	40	40	40	30	230
Flour	5		5				10
Butter (salted)	15	20	20	25	20	15	115
4-X cream	30	35	30	35	60	65	255
D-Zerta powder		3.5	3.5		3.5		10.5
Carrots	60						60
Tomatoes		100	100		100	100	400
Onions	80	80		80			240
Peas			60			60	120
Squash			100				100
Spinach				80	80		160
Orange juice	400	500	350	500	400	300	2450
Apple	100		100			100	300
Banana		40	40	40	140		260
Prune						20	20
Sugar	15	5	5	10	15	15	65
ESTIMATED DAILY TOTALS OF							
Protein	64.9	62.6	61.2	59.6	61.2	63.0	372.5
Carbohydrate	140.6	139.2	145.6	144.4	154.3	129.1	853.2
Fat	83.6	84.6	80.9	87.8	91.9	93.1	521.9
Calories	1626	1614	1602	1655	1737	1652	9886

Maximum number of items per diem, 14.
Minimum number of items per diem, 10.
Total number of items used, 19.

serving (Bassett, Elden and McCann, '31), then added to the pool. When a custard or other dish requiring the addition of several items was served, the raw food stuffs were assembled and the weight or volume of each item increased by an amount equal to one-quarter of the weight or volume

which the patient was to receive. After the necessary cooking, the total weight of the finished product was obtained in grams and this value divided by five. Since one-fifth of the total weight was equivalent to one-fourth of the amount actually given the patient, that amount was weighed as an aliquot and added to the pool. Obviously, only such items

TABLE 2

Low calcium diets. Raw food constituents (edible portion)

FOOD	RAW MEASURE—GRAM OR CUBIC CENTIMETER			TOTALS FOR 6 DAYS
	Day of period			
	1st and 4th	2nd and 5th	3rd and 6th	
Tenderloin	220		220	880
Round steak (as Hamburg)		75		150
Pork chops		120		240
Bread	40	20	20	160
Rice	20	10		60
Oatmeal		15		30
Farina			15	30
Potato	100	100	200	800
Cucumber	50		50	200
Tomato	100	140	160	800
Lettuce	40	40	40	240
Celery		20	20	80
Onion		20		40
Banana	100	100	50	500
Pineapple	100	100	150	700
Apple	100	100		400
Grape juice	100	200	100	800
Butter (salt free)	20	25	20	130
Mayonnaise	25	50	25	200
40 per cent cream	80	40	110	460
Walnuts			15	30
Sugar	25	15	20	120
Honey (strained)	50	30		160
ESTIMATED DAILY TOTALS OF				
Protein	49.1	50.0	52.8	303.8
Carbohydrate	200.9	192.5	144.3	1075.4
Fat	130.5	131.2	151.8	827
Calories	2243	2215	2221	13,358

Maximum number of items per diem, 19.
Minimum number of items per diem, 16.
Total number of items used, 23.

may be compounded in this way as will give a homogeneous mixture when cooked. An illustration may serve to clarify the apparent complexity of the procedure. Let it be assumed that the items used in the preparation of ice cream were:

	For patient	*For analysis*	*Total*
Milk	100.0 cc.	25.0 cc.	125.0 cc.
Egg	37.5 gm.	9.4 gm.	46.9 gm.
Sugar	6.2 gm.	1.5 gm.	7.7 gm.
Cream—40 per cent	25.0 cc.	6.2 cc.	31.2 cc.

The total for each item as given in the third column of the tabulation was the amount actually used in preparation of the ice cream. After cooking and freezing, the total weight of the assembled items, exclusive of the small amounts which had necessarily adhered to cooking utensils, was found to be 198 gm. From this net weight 39.6 gm. were saved for analysis and the remaining 158.4 gm. were served to the patient.

When the final meal of the period had been sampled, the contents of the collection jar were thoroughly mixed by stirring with a glass rod and the jar placed in an electric drying oven. The temperature of the oven was regulated so that it remained between 90 and 95° C. and a current of air was slowly drawn through it during the process of drying, thus preventing the condensation of any considerable amount of moisture upon its inner wall. As drying progressed, the diet sample was stirred from time to time to ensure as homogeneous a composition as possible. No attempt was made to dry to a constant weight, but after 3 or 4 days when the food seemed to be thoroughly dry, the sample was removed from the oven, pulverized in a porcelain mortar and returned to the original container. The gross weight of sample and container to 0.1 gm. was obtained and the powdered diet immediately transferred to a rubber stoppered glass bottle of suitable size where it was protected from the changing moisture content of the air of the room. The actual weight of the sample was obtained by difference after weighing the carefully washed and dried container.

Analytical methods

Large samples (60 to 70 gm.) were accurately weighed from the dry, pulverized diet and ashed in an electric muffle.[2] A solution of the ash in dilute hydrochloric acid was used for the determination of sodium, potassium, calcium, magnesium and phosphorus. Duplicate ashings were made only when the analysis of the original ashing gave an exceptionally high or low result. In such instances the questionable values were verified by repeating the analytical procedure using a duplicate ashing. The method of analysis, with the exception of the methods used in determination of potassium and chlorine and certain modifications of the iron method, has been described elsewhere (Bassett, Elden and McCann, '31). In the case of potassium, a gravimetric procedure (Van Alstine and Bassett, '34) was substituted in place of Shohl and Bennett's ('28) micro-volumetric method. Chlorine was determined by the method of Birner ('28).

Iron. Special care was taken to avoid contamination of the sample with iron. Approximately 100 gm. of the dried material were accurately weighed and ashed in an electric muffle at a temperature not exceeding 600° C. The ash was dissolved in a solution of pure hydrochloric acid diluted one-half with distilled water. After digestion for several hours on the steam bath to insure complete solution of the iron, the carbon residue was removed by filtration, washed and reashed. An extract of the ash thus obtained was made with hydrochloric acid and added to the original solution, which was finally concentrated and made to a volume of 100 ml. Twently ml. aliquots, containing 0.5 to 0.9 mg. of Fe, were pipetted into 100 ml. Florence flasks and further concentrated to a volume of 8 to 10 cc. When the contents of the flasks had cooled, two or three glass beads and 10 cc. of concentrated sulphuric acid were added to each. (The sulphuric acid was added while the neck of the flask was grasped by a small pair of crucible tongs and held in an inclined position. If this precaution is not taken, the reaction between the added acid and the contents of the flask may be so violent as to cause loss of material.) Each flask was then heated to boiling over a free flame while held in the inclined position and

[2] The temperature of the electric muffle used in the preparation of ash attained a maximum of 600° C. This is a somewhat higher temperature than is usually recommended when sodium or potassium is to be determined in ash (Peters and Van Slyke, '32).

agitated sufficiently to prevent bumping. With the beginning evolution of fumes of sulphuric acid the heat was increased and the process continued until all but 2 or 3 ml. of sulphuric acid had been expelled.[3] Finally 30 ml. portions of 20 per cent sulphuric acid were added to the contents of each flask, the mouths covered with a watch crystal and the flasks placed on the steam bath for 6 hours.

Boiling with concentrated sulphuric acid was found to insure complete oxidation of the iron without addition of any oxidizing agent likely to interfere with the subsequent titration. In the final stages of concentration of the solution some ferric sulphate and calcium sulphate precipitated. The ferric sulphate was soluble with some difficulty in 20 per cent sulphuric acid, hence the long period of digestion on the steam bath. Reference to the actual method of titration has been given in the first paper (Bassett, Elden and McCann, '31).

Analysis of data

Dunn ('29) has shown that statistical methods may be applied effectively to even as small a series of observations as were obtained in these period by period analyses of the food, provided that the limitations of such an application be kept in mind. Table 3 presents in a condensed form the result of such statistical analysis as the data appear to warrant. The mean, its standard deviation, and coefficient of variation have been computed for each element determined in the two series of diets. Calcium and iron are the mineral constituents showing the greatest variation in each series. The least variable element is potassium, as found in the low calcium diet. In table 4 the means determined by analysis are compared with those calculated from Sherman's tables. In the case of the high calcium diet, one notes that the values of nitrogen, sodium, potassium and magnesium, as determined by analysis, are in fair agreement with their calculated values, while the amounts of calcium, phosphorus, chlorine and iron determined differ considerably from the amounts calculated. The means of the series of analyses made of the low calcium diet agree fairly well with the calculated values in the case

[3] The digestion with sulphuric acid must be performed in a well-ventilated hood.

TABLE 3

Mean values of mineral constituents of high and low calcium diets calculated from period by period analysis of the food

ELEMENT	HIGH CALCIUM DIET[1]			LOW CALCIUM DIET[2]		
	Mean	Standard deviation	Coefficient of variation	Mean	Standard deviation	Coefficient of variation
	Grams in 6 days	Grams	Per cent	Grams in 6 days	Grams	Per cent
Nitrogen	62.16 ±0.394	1.85	2.9	55.97 ±0.296	1.32	2.3
Sodium	6.89 ±0.047	0.22	3.2	3.37 ±0.018	0.08	2.3
Potassium	17.74 ±0.13	0.61	3.4	15.52 ±0.029	0.13	0.8
Calcium	7.01 ±0.11	0.53	7.5	0.91 ±0.018	0.08	8.8
Magnesium	1.363 ±0.007	0.036	2.6	1.178 ±0.007	0.033	2.8
Chlorine	11.10 ±0.08	0.40	3.7	6.07 ±0.083	0.37	6.1
Phosphorus	8.61 ±0.09	0.44	5.1	4.28 ±0.029	0.13	3.0
Iron	0.0514±0.0008	0.0039	7.6	0.0577±0.0011	0.0049	8.6

[1] Each mean is calculated from the results of analysis of food in eleven consecutive metabolic periods.

[2] As above, except that in this instance there were only ten consecutive metabolic periods.

TABLE 4

Mean values of mineral constituents computed from analysis of food are compared with mean values computed by use of Sherman's tables

ELEMENT	HIGH CALCIUM DIET			LOW CALCIUM DIET		
	Mean found by analysis	Mean calculated from Sherman	Difference between mean found and calculated	Mean found by analysis	Mean calculated from Sherman	Difference between mean found and calculated
	Grams in 6 days	Grams in 6 days	Per cent	Grams in 6 days	Grams in 6 days	Per cent
Nitrogen	62.16	59.68	+ 2.4	55.96	48.64	+15.0
Sodium	6.89	6.80	+ 1.3	3.37	3.55	— 5.0
Potassium	17.74	17.65	+ 0.5	15.52	18.00	—13.8
Calcium	7.01	7.76	— 9.7	0.91	1.35	—32.6
Magnesium	1.363	1.313	+ 3.8	1.179	1.170	+ 0.77
Chlorine	11.10	9.99	+11.2	6.07	5.83	+ 4.1
Phosphorus	8.61	7.81	+10.2	4.28	4.55	— 5.9
Iron	0.0514	0.0676	—24.0	0.0577	0.0653	—11.6

of sodium, magnesium, phosphorus and chlorine. All others show a difference between found and calculated means which is greater than 10 per cent.[4]

An approximation of the excess of acid or base in the ash of the two diets (derived from the mean values of acidic

TABLE 5

Acid-base components of high calcium and phosphorus diet, milliliters of 0.1N acid and base per 6-day period

BASE	MEAN VALUE IN GRAMS	MEAN VALUE ML. 0.1N SOLUTION	ACID	MEAN VALUE IN GRAMS	MEAN VALUE ML. 0.1N SOLUTION	EXCESS BASE ML. 0.1N SOLUTION
Na	6.89	2995	Cl	11.10	3130	
K	17.74	4537	S[1]	4.81	3000	
Ca	7.01	3505	P[2]	8.61	4999	
Mg	1.363	1120				
Totals		12,157			11,129	1028

[1] Mean value for sulphur calculated from Sherman.

[2] Base equivalent of phosphorus calculated to pH7.4 (ml. 0.1M $H_3PO_4 \times 1.8$).

TABLE 6

Acid-base components of low calcium diet milliliters of 0.1N acid and base per 6-day period

BASE	MEAN VALUE IN GRAMS	MEAN VALUE ML. 0.1N SOLUTION	ACID	MEAN VALUE IN GRAMS	MEAN VALUE ML. 0.1N SOLUTION	EXCESS ACID ML. 0.1N SOLUTION
Na	3.37	1465	Cl	6.07	1711	
K	15.52	3969	S[1]	5.83	3646	
Ca	0.91	455	P[2]	4.28	2484	
Mg	1.178	968				
Total		6857			7841	984

[1] Mean value for sulphur calculated from Sherman.

[2] Base equivalent of phosphorus calculated to pH7.4 (ml. 0.1M $H_3PO_4 \times 1.8$).

and basic constituents) is given in tables 5 and 6. From these it appears that the ash of the high calcium diet is alkaline, and of the low calcium diet acid. However, attention must be called to the fact that the sulphur content of the food was computed from tables of averages (Sherman, '26).

[4] These results do not detract from the general usefulness of Sherman's tables. Differences between means found and calculated would probably be as great if any other set of average tables were substituted in their place.

The difference in each case is about 1 liter of 0.1N base or acid. If this difference is due to error introduced by estimation of the sulphur content of the food, and if it be assumed, for example, that the diets actually had a nearly neutral ash, then in the first instance (table 5) the calculated value assigned to sulphur is about 33 per cent too low and in the second instance (table 6) about 27 per cent too high. While the actual sulphur content of the food may vary from the calculated to the extent indicated (see table 4 for examples), such a variation is probably unlikely. It is apparent, however, that no definite statement regarding the acidity or alkalinity of the ash of these diets may be given without more accurate knowledge of their sulphur content.

DISCUSSION

Number of items on menu

Reference to tables 1 and 2 shows that nineteen different foods were used in the high calcium diet and that twenty-three foods were used in the low calcium diet. Granting that simplicity in a diet is desirable for metabolic work, these diets appear to include an unnecessarily large number of items. On the other hand, the effect of an extremely monotonous diet on the subject of the experiment must be considered. Such a diet may be comparatively well tolerated by children, but when given to adults frequently leads to refusal or smuggling of food.

The importance of good cooking and attractive menus has been emphasized by Gephart and Du Bois ('15). With the diet rigidly limited to four or five foods, the serving of attractive well-cooked meals becomes a serious problem, if not an impossibility. The fact that the menus listed in table 1 were, in a certain instance, repeated every 6 days for approximately 4 months and were well tolerated is an argument in favor of a liberal diet.

Evaluation of technical errors

The following experiment was performed in order to determine whether the failure to obtain uniform results in period by period sampling of the food depended upon errors inherent in the methods employed or was due to actual variations in the mineral content of the foods served in these periods.

Unused portions of the dried samples of food from the high calcium diet were pooled and thoroughly mixed together.

TABLE 7

Consecutive analyses of a single specimen of dried food showing variations due to technical errors

ELEMENT	NUMBER OF SAMPLES ASHED	WEIGHT OF EACH SAMPLE IN GRAMS	MEAN IN GRAMS[1]	STANDARD DEVIATION IN GRAM	COEFFICIENT OF VARIATION
Sodium	10	60	7.09 ±0.021	0.096	1.36
Potassium	10	60	18.42 ±0.068	0.305	1.65
Calcium	10	60	6.87 ±0.015	0.066	0.96
Magnesium	10	60	1.388±0.007	0.033	2.37
Chlorine	20	1	11.11 ±0.046	0.296	2.66
Phosphorus	10	60	8.46 +0.031	0.136	1.61

[1] Each mean has been computed on the basis of a 6-day period.

When the mixture appeared to have become neither more nor less homogeneous than the dried food sample of a single period, ten consecutive portions of 60 gm. each were weighed out and ashed. The ten samples of ash thus prepared were subjected to the routine methods of analysis. In addition, twenty smaller samples of 1 gm. each were used for the determination of chlorine. The data obtained have been summarized in table 7.[5] Strictly speaking, these data can be compared only with the period by period analyses of the high calcium diet (table 3), but it is reasonable to suppose that consecutive analyses of a similar specimen of food prepared from the low calcium diet would yield comparable results. From the tabulation it is evident that the standard deviations

[5] A statistical evaluation of the amounts of nitrogen and iron in consecutive samples taken from the same specimen of food has not been made.

are, for the most part, smaller than those found in the period by period analysis of the food. Comparatively large fluctuations in the amounts of calcium and phosphorus no longer occur. The results obtained in the cases of sodium and potassium are somewhat less convincing, although when compared with the previously cited data (table 3) for the high calcium diet it is found that the respective standard deviations have been decreased about 50 per cent. Magnesium, on the other hand, showed nearly as much variability in the series of analyses of the single specimen of dried food as was found in period by period analysis. Chlorine showed more variability than any of the other elements. However, previous experience leads us to attribute much of the variation in this instance to lack of uniformity among small samples. While the data do not permit a differentiation between errors introduced by sampling and those introduced by analytical methods and manipulations, they show the gross technical errors exclusive of errors which may be introduced in the diet kitchen. Unfortunately, the latter are not readily susceptible to statistical measurement. Since we have been unable to detect any gross inaccuracy in the initial measurement of samples of food by the dietitian, the assumption has been made that the larger deviations of sodium, potassium, calcium and phosphorus from their respective means (as found in period by period analysis of food) actually represent fortuitous variations in the concentrations of these elements in the food of different periods. If this is so and one is to avoid errors of unknown and perhaps considerable magnitude, it becomes necessary to sample and analyze the food given in every metabolic period. When the same diet formula is repeated many times in consecutive metabolic periods, and analyzed in each by the methods described, mean values of chlorine, magnesium and probably of nitrogen may be computed, and these values used as an approximation of the true intake in each period. Where calcium, sodium, potassium and phosphorus are concerned, it seems advisable to compute the intake in each period from analysis of the sample of food collected in that period.

The limited data available in the case of iron do not permit us to submit statistical evidence from which a probable differentiation can be made between technical errors and actual changes in the iron content of the food.

SUMMARY

1. A method for determination of mineral intake in human metabolic experiments has been described in detail.

2. The method has been applied to determine the amount of nitrogen, sodium, potassium, magnesium, calcium, phosphorus, chlorine and iron in, a) high calcium diet, b) low calcium diet.

3. The mineral content of the high calcium diet was determined during eleven consecutive metabolic periods of 6 days each, and of the low calcium diet during ten consecutive periods of 6 days each.

4. Statistical methods have been employed in an attempt to determine the magnitude of the technical errors of the procedure and to differentiate between such errors and actual variations in the mineral content of the food mixture.

5. The implications of the statistical analysis of the data have been discussed in the text.

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THE RELATION OF MANGANESE TO CONGENITAL DEBILITY

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The high percentage of deaths among the young of rats receiving milk fortified with copper and iron, in comparison with the vigor and physical well-being of the young of rats receiving the same diet to which manganese was added has led to an inquiry concerning the cause of the infant mortality among the former group. Do the young die of congenital debility, the result of too little manganese in the diet of the mother during gestation, or is there a failure in lactation due to the lack of mammary gland development, as suggested by Orent and McCollum ('31)?

The hypothesis of a vitamin B deficiency as the cause of death of the young of milk-fed rats, postulated by Sure ('24, '29) would seem to be untenable, since rats receiving milk to which copper, iron and manganese were added in adequate amounts have been raised through five generations.[1] The vigor and weaning weights of the young would seem to attest to the efficiency of the diet. The explanation of the beneficial effect of the larger amounts of vitamin B extracts and concentrates used by Sure in lactation experiments with rations consisting of 50 per cent milk powder would seem to be either contamination with manganese of the materials tested or considerable destruction of vitamin B in the milk powder, since the nutritive ratio of the milk powder ration used by Sure is approximately the same as that of Guernsey

[1] Unpublished data.

milk in our experiments and therefore the B_1 of the two rations should be similar. It is interesting to note that Sure found a 25 per cent alcoholic extraction of wheat embryo much more potent as a source of vitamin B for lactation than a 75 per cent extraction. It is probable that the 25 per cent extract contained significant amounts of manganese.

The importance of manganese in nutrition, more especially in relation to reproduction, has been demonstrated by Orent and McCollum ('31), Skinner, Van Donk and Steenbock ('32) and Kemmerer, Elvehjem and Hart ('31). Too little manganese results in testicular degeneration and sterility after about the 150th day. Young born of females fed manganese-free diets die either at birth or shortly after. Spectrographic analysis of the ash of the young indicates that the manganese is in low concentration and suggests that the young were not 'well-born.' On the other hand, the mothers of these manganese-low young manifest little interest in their offspring, giving them scant opportunity to suckle. Examination showed little mammary gland development (Orent and McCollum, '31). The young, therefore, may have obtained insufficient nourishment. In order to test this point, Orent and McCollum transferred young of mothers fed manganese-free diets to stock mothers and young of stock mothers to the manganese-free mothers. Of the 107 young transferred to stock mothers, only one was raised, with a weaning weight much below the average. In two instances stock young given to mothers receiving manganese-free diets were slightly more successful. One mother raised two stock young of the seven which were substituted for her own; and the other, five out of seven. The weaning weights of these conformed to the average of stock young. The failure of the stock animals to raise the young of manganese-low mothers was the more notable, since, in general, lactating rats are exceedingly open-minded on the subject of adoption and will accept and suckle young of other animals with little question.

Orent and McCollum's results, tentatively interpreted as indicating failure in lactation of the manganese-low mothers,

seemed rather to point to congenital debility of the young, or possibly to too little vitamin B in the ration used. In order to test this point with milk-fed animals, the investigation herein reported was undertaken.

EXPERIMENTAL PROCEDURE

Two groups of animals, one at 11 days and a second at 8 weeks of age, consisting of four females and two males in each group, were transferred from stock to the experimental laboratory and given a ration consisting of boiled Guernsey milk to which were added 0.8 mg. of copper, 5 mg. of iron and 2 drops of 2 per cent potassium iodine solution to every 100 cc. of milk. For purposes of comparison other groups were tested: one, a fourth generation to receive the milk, copper and iron ration supplemented with 1.5 mg. of manganese per 100 cc. of milk; and others, consisting of animals receiving our stock ration with iron, copper and manganese additions.[2] When the young of these various groups were born, one of two procedures was followed: 1) The young shortly after birth were prepared for analysis of the manganese content. 2) The young were exchanged, manganese-low young to stock mothers and stock young to manganese-low mothers, thus giving an opportunity to determine whether it is possible for the mothers receiving the milk, copper and iron diet to suckle vigorous stock young or for the manganese-low young to survive if sufficient food is available. Theoretically, the quality of the milk produced by the respective mothers should not differ in the amount of manganese contained therein, since, according to Kemmerer and Todd ('31, '32), it is not possible to increase the manganese content of milk by feeding. Under normal conditions, the manganese content of cow's milk is low, differs with the individual, and cannot be increased by feeding. Nothing is known directly regarding the manganese content of rats' milk. It seems

[2] The stock ration consisted of a mixture of dried cooked soy beans, yellow corn, sodium chloride and calcium carbonate. Pasteurized Holstein milk with 0.4 mg. of Cu, 0.75 mg. of Mn., 2 mg. of Fe and 1.5 mg. of I per 100 cc. was also given ad lib.

probable, however, that similar factors control milk production in both species.

In the lactation tests six of a given litter were transferred to the foster mother as soon after birth as possible. The efficiency of the mother as a milk producer was judged by the number of young successfully suckled, the weaning weight of the young, and the weight gain or loss of the mother during lactation. A record of the milk consumption of the mothers receiving the two milk diets was kept in order to determine if failure in lactation was caused by a possible low food ingestion. The physiological well-being of the young at birth was judged by the viability and the gain in weight during the suckling period.

In order to obtain further information regarding the high mortality of the young of the milk-fed groups, comparisons were made of the manganese content at various ages from weaning to sexual maturity of animals receiving the milk ration with and without the manganese additions. For these tests, a few days after weaning, nine young were placed on the milk-copper-iron ration and nine on the milk ration with the manganese addition. Three of each group subsequently were taken for analysis at intervals of approximately 2 weeks, the milk-manganese animals being continued 2 weeks longer than the milk, copper and iron group.

In preparation for analysis, after anesthetizing all animals were washed with distilled water, the alimentary tracts were removed, the bodies were dried, subsequently ashed, and the manganese determined according to the method of Skinner and Peterson ('30). To obtain sufficient material for satisfactory tests with the newborn animals it was found necessary to use at least two litters; whereas with the older animals one animal was found sufficient. In two cases comprising the young of manganese-low animals, known amounts of manganese were added in order to obtain more satisfactory determinations and thus check the reliability of the results with other low manganese young.

Eight first generation females receiving the milk-copper-iron diet, produced in the time allowed at least 103 young; 47 in first litters, 33 in second, and 23 in third. All of the third litters and some of the second were sired by stock males. Since in some instances the young were born during the night, more may have been born than were seen; indeed, there was evidence that such was the case. The data, however, pertain only to such young as were intact. Of these, 41 either were born dead or died within two hours, 25 were anesthetized for subsequent manganese analysis, and 37 were transferred to 7 stock mothers. Sixty-nine per cent of these subsequently died within a few days; 12 were raised by 2 females (6 each) to weaning weights of 53.7 and 41.5 gm., respectively.

The mortality record of the young of these low-manganese females is quite different from that of the young of the fifth generation females receiving the milk diet with added manganese, or of the stock animals. In the milk-manganese groups, 100 per cent of the young were reared with an average weaning weight at 28 days of 69.2 gm., and in the stock groups 98.6 per cent were raised with an average weaning weight of 70.1 gm. (table 1).

The low-manganese mothers were more successful in suckling the young of stock than their own offspring (table 1). Eleven litters of six young each of stock mothers were given to mothers receiving the low-manganese diet. In five instances, these low-manganese mothers had lost all their young at birth; in five, part of the litter had died and the remainder which were given to stock mothers to raise, subsequently died. Four of the groups transferred from stock replaced first litters of the low-manganese mothers; four, second litters; and three, third litters. Notwithstanding the high mortality of the young of the low-manganese mothers, these animals were able to suckle successfully 96.9 per cent of their foster young to weaning weights (average 58.7 gm.) which compared favorably with those of the milk-manganese mothers and not far below those of the young of the stock mothers reported. There was little difference in the lactation tests

TABLE 1

Relation of manganese content of the diet to lactation

RAT NO.	AGE WHEN PLACED ON DIET	LITTER SEQUENCE	AGE OF MOTHER AT BIRTH OF YOUNG	NUMBER OF YOUNG BORN	WEIGHT AT BIRTH	VIABLE YOUNG	MN-LOW YOUNG ADOPTED BY STOCK	STOCK YOUNG ADOPTED BY MN-LOW MOTHERS	YOUNG RAISED	WEIGHT OF YOUNG 28 DAYS	WEIGHT GAIN OR LOSS OF MOTHER 28 DAYS
Diet: Milk + 0.8 mg. Cu + 5 mg. Fe + 2 d. 2 per cent K.I. sol. + 1.5 mg. Mn per 100 cc.											
			Weeks		*gm.*	*Per cent*	*Number*	*Number*	*Per cent*	*gm.*	*Per cent*
50	4th gen.	1	14.5	6	7.1	100	..	..	100	63.3	— 2.7
44	4th gen.	1	15.0	3 [2]	6.8	100	..	..	100	76.0	— 3.9
45	4th gen.	1	14.8	5 [2]	6.6	100	..	..	100	74.1	— 5.8
43	4th gen.	1	15.5	10	6.0	100	..	..	100	76.0	+ 1.3
42	4th gen.	1	15.9	9	4.8	100	..	..	100	56.6	— 4.5
Average			15.1	6.6	6.4	100	..	..	100	69.2	— 3.1
Diet: Stock food + manganese											
11	.....	..	...	7	9.6	100	..	..	100	73.6	— 6.8
12	.....	..	...	6	7.6	100	..	..	100	72.1	— 0.0
13	.....	..	...	11	9.0	100	..	..	100	60.0	— 2.8
14	.....	..	...	10	6.2	90	..	..	90	65.5	— 3.6
16	.....	..	...	9	6.6	100	..	..	100	82.2	— 5.0
17	.....	..	...	8	7.5	100	..	..	100	75.6	— 6.0
18	.....	..	...	9	9.3	100	..	..	100	66.3	—11.0
Average				8.6	8.0	98.6	..	..	98.6	70.1	— 5.0
Diet: Milk + 0.8 mg. Cu + 5 mg. Fe + 2 d. 2 per cent K.I. sol.											
1	8 weeks	1	14.0	7	4.8	0	..	..	...	...	...
2	8 weeks	1	17.0	9	5.1	[1]	..	6	66	58.5	— 7.6
3	8 weeks	1	13.0	7	3.8	43	3 [3]	..	...	...	...
5	11 days	1	16.0	8	5.1	[1]	..	6	100	65.6	+ 2.7
6	11 days	1	16.0	3	5.3	0	..	..	...	...	...
7	11 days	1	18.0	5	6.8	0	..	6	100	60.7	+ 1.4
8	11 days	1	19.0	8	5.6	75	6 [4]	6	100	56.5	+ 1.4
Average			16.1	6.7	5.2	...	..	...	91.5	60.5	— 0.52
1	8 weeks	2	20.0	7	5.3	85.7	6 [3]	6	100	69.0	+ 5.7
2	8 weeks	2	22.0	8	4.9	[1]	..	6	100	49.2	— 2.7
5	11 days	2	27.0	5	5.0	0	..	6	100	49.0	—16.0 [6]
6	11 days	2	21.0	9	5.0	77.7	7 [3]	..	...	...	...
7	11 days	2	28.0	4	6.0	0	..	6	100	65.0	± 0.0
8	11 days	2	...	0	..	...	..	..	...		
Average			23.6	5.1	5.2	...	..	..	100	58.0	— 3.2
1	8 weeks	3	30.0	7	5.4	85.7	6 [3]	6	100	60.7	+ 3.4
2	8 weeks	3	32.0	8	4.4	75	6 [5]	6	100	51.8	+ 3.1
6	11 days	3	28.0	8	5.5	37.5	3 [3]	6	100	60.1	— 2.7
Average			30.0	7.6	5.1	...	..	..	100	57.5	— 1.3

[1] Anesthetized.

[2] Died.

[3] Young of other milk-Mn groups added to make litter of six.

[4] Weaning weight 28 days—53.7 gm.

[5] Weaning weight 28 days—41.5 gm.

[6] Diarrhoea during excessive heat.

of the animals which were placed on the low-manganese diet at 11 days (animals 5, 6, 8, table 1) and 60 days (animals 1, 2, table 1) of age, respectively.

The manganese content of the young of stock (average 0.002 mg.) and of mothers receiving the milk ration fortified with copper, iron and manganese (average 0.0026 mg.)

TABLE 2

Relation of the diet of the mother to the manganese content of the young at birth

RAT NO.	DIET	AGE OF MOTHER AT BIRTH OF YOUNG	LITTER SEQUENCE	NUMBER OF YOUNG BORN	WEIGHT AT BIRTH	NUMBER OF YOUNG ANALYZED	MANGANESE	
							Per Animal	Per kilogram moist weight
		Weeks			*gm.*		*mg.*	*mg.*
60	Milk-Cu-Fe-Mn	13.0	1	9	4.9)	14	0.0028	0.526
64	Milk-Cu-Fe-Mn	13.0	1	5	6.0)			
65	Milk-Cu-Fe-Mn	13.5	1	6	5.2)	15	0.0024	0.502
66	Milk-Cu-Fe-Mn	13.5	1	9	4.8)			
11	Stock food-Mn	12.0	1	6	6.5)	19	0.0019	0.328
12	Stock food-Mn	14.0	1	13	5.5)			
18	Stock food-Mn	14.0	1	7	5.7)	14	0.0024	0.407
19	Stock food-Mn	14.0	1	7	5.6)			
8	Stock food-Mn	...	3	11	6.2	11	0.0023	0.370
1[1]	Milk-Cu-Fe	14.0	1	7	4.8)			
2[1]	Milk-Cu-Fe	17.0	1	9	5.1)	21	0.0007	0.147
3[1]	Milk-Cu-Fe	13.0	1	7	3.8)			
5[2]	Milk-Cu-Fe	16.0	1	8	5.1)	13	0.0006	0.124
7[2]	Milk-Cu-Fe	18.0	1	5	6.8)			
1[1]	Milk-Cu-Fe	20.0	2	7	5.3)	15	0.0006	0.107
2[1]	Milk-Cu-Fe	22.0	2	8	4.9)			

[1] Placed on diet of milk-Cu-Fe at 8 weeks of age.

[2] Placed on diet of milk-Cu-Fe at 11 days of age.

differed only slightly when estimated on the basis of the amount per animal (table 2). Estimations of the manganese content per kilogram of moist weight were somewhat higher for the milk-manganese group than for the stock group; 0.513 mg. per kilogram for the former, and 0.363 mg. per kilogram for the latter. The manganese content of the young

of rats receiving the same milk ration without the manganese, estimated on moist weight, was 75 per cent lower than those of the milk-manganese group and 65 per cent lower than the stock group. Young of mothers receiving the manganese-deficient diet at 11 days contained only slightly less than those which were placed on the diet somewhat later. Second litters of the manganese-deficient mothers also contained slightly less than first litters.

An estimation of the daily ingestion of manganese by the three groups during gestation shows a parallelism between ingestion and storage during fetal life. Animals receiving the milk, copper and iron ingested an average of approximately 0.022 mg. of manganese per day, whereas those receiving the same diet with added manganese and the stock diets obtained 1.202 mg. and 0.816 mg. per day, respectively.

The causes of the loss or gain in weight during lactation are little understood. Sure has related loss in weight of mothers receiving milk diets to the low vitamin B of the milk. In the milk-fed animals herein reported the weight gain or loss of the mothers on the various diets during lactation did not differ materially. Omitting the animal which lost 16 per cent during an alimentary tract infection manifested by diarrhoea, the mothers receiving the low-manganese diet lost slightly less than any other group studied including the stock animals (table 1). These data, therefore, seem to add further evidence that milk does not supply too little vitamin B for the lactating mother and the needs of six sucklings. The amount of milk taken by the animals during lactation on the two types of milk modification did not differ materially (table 3). Manganese, therefore, would seem to bear no relation to appetite, again indicating that the infant mortality of milk-fed rats is not due to a vitamin B deficiency.

The manganese content of the young of the stock animals at birth averaged 0.002 mg. per animal, or 0.363 mg. per kilogram of moist weight. Shortly after weaning (33 to 36 days) while the amount for a given animal had increased to 0.013 mg., the amount estimated on the basis of weight

(average 0.153 mg. per kilogram) had decreased considerably (table 4). From weaning on, the animals receiving the milk-copper-iron ration continued to store small amounts of manganese, the amount being directly proportional to their increase in weight so that estimated on the basis of weight

TABLE 3

Relation of manganese to milk ingestion during lactation

RAT NO.	LITTER SEQUENCE	LACTATION PERIOD: 21 DAYS			YOUNG RAISED	
		Weight of mother average	Weight gain or loss	Milk consumed per day	Number	Weight at 21 days
Diet: Milk + 0.8 mg. Cu + 5 mg. Fe + 2 d. 2 per cent K.I. sol. + 1.5 mg. Mn per 100 cc.						
Third generation						
		gm.	*Per cent*	*cc.*		*gm.*
36	1	164	— 5.7	89	6	22.2
41	1	190	— 5.7	105	5	35.2
41	2	226	— 6.5	99	6	33.3
37	1	194	— 2.5	125	6	34.1
37	2	240	— 3.7	130	6	44.5
79	1	170	— 4.1	107	6	31.8
79	2	238	+ 0.8	111	5	32.0
Fourth generation						
44	1	220	— 4.1	126	6	44.3
45	1	206	— 3.1	115	6	41.5
43	1	225	+ 1.0	162	6	45.8
42	1	207	— 8.0	112	6	34.6
40	1	207	— 5.6	126	6	40.0
Diet: Milk + 0.8 mg. Cu + 5 mg. Fe + 2 d. 2 per cent K.I. sol.						
1	2	218	+ 0.6	152	6[1]	43.3
1	3	228	+ 4.7	136	6[1]	41.6
2	1	193	— 0.0	60	4[1]	33.0
2	2	213	— 0.8	77	6[1]	28.0
2	3	218	— 0.9	77	6[1]	31.3
5	1	190	+ 1.6	107	6[1]	38.0
5	2	203	—13.0[2]	87	6[1]	31.3
6	3	218	— 2.7	97	6[1]	34.8
7	1	204	+ 0.5	108	6[1]	38.3
7	2	232	+ 0.8	152	6[1]	48.3
8	1	195	— 0.0	88	6[1]	30.0

[1] Young transferred from stock.

[2] Diarrhoea during excessively hot weather.

the amount contained in a given animal was approximately the same at 33 to 36 days and at 61 to 63 days, namely, 0.139 mg. and 0.142 mg. per kilogram, respectively.

With the animals which received the milk-manganese ration, the results were quite different. In the first 2 weeks after weaning, there was a rapid storage, five-fold and over when estimated on the basis of the individual animal, and three-fold estimated on the basis of moist weight. After this initial rapid storage, the amount remained constant at approximately 0.080 mg. per animal, or 0.40 mg. per kilogram till the seventy-eighth day, the beginning of the gestation period.

TABLE 4

The relation of diet to the manganese content of the young at various ages

DIET	AGE	NUMBER OF ANIMALS	WEIGHT AVERAGE	MANGANESE PER ANIMAL		MANGANESE PER KILOGRAM	
				Average	Range	Average	Range
	Days		*gm.*	*mg.*	*mg.*	*mg.*	*mg.*
Stock	Birth	44	5.8	0.002	0.002–0.002[1]	0.363	0.328–0.406
Milk-Cu-Fe	33–36	3	88.1	0.013	0.013–0.014	0.153	0.146–0.165
Milk-Cu-Fe	47–50	3	127.6	0.018	0.017–0.019	0.139	0.121–0.161
Milk-Cu-Fe	61–64	3	144.6	0.020	0.018–0.022	0.143	0.110–0.162
Milk-Mn-Cu-Fe	47–50	3	139.5	0.079	0.072–0.082	0.573	0.475–0.686
Milk-Mn-Cu-Fe	61–64	3	192.3	0.083	0.080–0.086	0.404	0.391–0.503
Milk-Mn-Cu-Fe	73–75	3	220.0	0.088	0.087–0.088	0.399	0.389–0.407

[1] The manganese content of the young of stock animals, analyzed in groups of 19, 14 and 11, averaged 0.0019, 0.0023 and 0.0022 mg. per animal, respectively.

COMMENT

The results of the investigation point to the conclusion that the high mortality of the young of animals receiving the milk-copper-iron diet is due to congenital debility directly related to a deficiency of manganese in the diet of the mother during gestation. The young, although of normal size at birth, are not sufficiently robust to survive the ordeal of birth; the few which do survive are not strong enough to suckle. It would seem, therefore, that manganese is an essential constituent of the developing organism. If the diet of the mother during gestation is adequate, the young are born with a store of manganese which is surprisingly constant. The amount

per animal at birth of our stock groups (average 0.002 mg.) as well as those receiving the milk-manganese diets (average 0.003 mg.) was found to be within the limits of error of that reported by Skinner, Peterson and Steenbock ('31) in the young of rats receiving their stock diet with the manganese addition.

During suckling there is considerable increase in the manganese store, suggesting that the milk of the rat is somewhat higher than that of the cow, since rats receiving cow's milk without manganese subsequent to weaning store little. Following weaning, storage of manganese takes place rapidly if sufficient is contained in the diet. After 2 weeks on the milk supplemented wtih 1.5 mg. of manganese per 100 cc., the amount of manganese per animal (average 0.079 mg.) was found to be only slightless less than that found 4 weeks later (average 0.088 mg.). Following the initial increase, the amount stored, estimated on body weight, had decreased 30 per cent in the 4 weeks of the study. This percentage decrease suggests that storage was confined to certain tissues rather than to the body as a whole, the tissues most avid for manganese being, according to Skinner, Peterson and Steenbock, bone, brain, hide, kidney and liver.

Although failure in lactation may occur in animals raised on diets quite free from manganese, as suggested by Orent and McCollum, adequate lactation is possible on a diet which contains too little manganese for the development of normal young in utero. Cow's milk containing 0.03 mg. per liter (the Guernsey milk used) supplies sufficient manganese for lactation, since animals receiving this low-manganese diet from infancy were able to suckle successfully six vigorous foster young. In one case three successive foster litters were raised by a rat receiving milk modified with only iron and copper.

The addition of manganese to the milk diet was without influence on the milk consumed by the rats during lactation. This was reflected in the weaning weights of the young and the consistency of the weights of the mother receiving the milk ration during lactation.

SUMMARY

In an endeavor to determine the cause of the high infant mortality of milk-fed rats, reproduction, lactation and the manganese content of the young at birth, at weaning, and thereafter at various periods to sexual maturity have been compared in animals receiving Guernsey milk plus copper and iron, with those receiving the same ration fortified with manganese, as well as with stock fed animals. The lactating ability of the animals receiving the simple milk ration was specifically tested by exchanging their young for vigorous stock young. In a number of instances where the young of the milk-manganese low animals died, they also were given stock young to raise. Records were kept of the amount of milk consumed by each animal during lactation.

The results of the investigation point to the following conclusions:

1. The high percentage of deaths of the young of animals reared on milk diets modified with copper and iron is due to congenital debility, the result of too little manganese in the diet of the mother. Young born of these milk-fed animals were found to contain 65 per cent less manganese than the young of animals receiving a similar ration with added manganese.

2. Animals reared from weaning on a milk diet with no added manganese are able to suckle vigorous normal young to average weaning weights comparable to those of stock young or of young receiving milk with manganese added.

3. Storage of manganese in the young during the suckling period is greater than subsequently on a cow's milk ration, but less than during a subsequent period on a milk ration with 1.5 mg. of manganese per 100 cc. of milk, suggesting that rat's milk is higher in manganese than the Guernsey milk tested.

4. The milk ingestion of rats during the lactation period is not influenced by the amount of manganese the milk contains. Rats receiving the simple milk ration consumed as much milk as those receiving the milk-manganese ration.

5. The Guernsey milk tested supplied enough vitamin B for the lactating mother and the growth needs of six young.

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RESPIRATORY METABOLISM STUDIES IN HYPERINSULINISM FOLLOWING INGESTION OF GLUCOSE [1]

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Hyperinsulinism is now a well-recognized clinical entity. There is, however, as yet, much to be known with regard to the conditions which lead to, and which may result from, this disturbance. It was, therefore, considered of interest to investigate the respiratory metabolism, in order to determine whether such studies might reveal some as yet unrecognized metabolic defect. As will presently be shown, respiratory metabolism studies are also of value otherwise. They may be of diagnostic value since, in the interpretation of blood sugar time curves in suspected cases, they help to differentiate between apparent and real failure of absorption of glucose from the gastro-intestinal tract. The literature of hyperinsulinism has been repeatedly reviewed and, for purposes of brevity, reference will be made to relevant facts only.

Though hypoglycemia was produced experimentally many years ago and has been known to exist in a variety of clinical disorders, no signs nor symptoms, nor disturbances noted experimentally, were ever attributed to lowering of blood sugar until the discovery of insulin. Experiences with insulin shock in diabetes led to the recognition of a symptom complex identical with insulin shock, but independent of insulin injec-

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tions. Harris ('24) first drew attention to this condition to which he gave the name hyperinsulinism.

As the fundamental disturbance is reduction of blood sugar, it is obvious that the term hyperinsulinism may at times be a misnomer, for hypoglycemia may be produced in a variety of ways other than by excess production of insulin. Hypofunction of the thyroid, adrenals and anterior pituitary bodies are examples. As the liver is also intimately concerned with the regulation of blood sugar, a variety of disorders of this organ may also lead to hypoglycemia. As is well known, hypoglycemia may be produced in the experimental animal by hepatectomy and administration of a variety of liver poisons (phosphorus, chloroform, etc.). Clinically, a variety of conditions cause destruction of liver tissue and thus tend to simulate, metabolically, experimental hepatectomy (carcinoma, acute yellow atrophy, etc.). Wauchope ('33), in an excellent review, discussed the morbid anatomy of hypoglycemia and classified symptoms according to organs affected. It would thus appear that, for purposes of exact terminology, hyperinsulinism should be confined to conditions in which there is definite proof of production and liberation of insulin in excess of the normal. The case reported by Wilder et al. ('27) may be cited as an example of true hyperinsulinism; operation and autopsy showed a carcinoma of the pancreas which originated from the islet cells. A large yield of insulin was obtained from the metastases in the liver. The term, hyperinsulinism, might thus also be applied when symptoms are associated with adenomata of the islet cells of the pancreas. Surgical removal of such adenomata has resulted in cure of the symptoms. All other cases of hypoglycemia in which no definite proof of cause can be found might be classified as spontaneous, or idiopathic, hypoglycemia. Such classification is, however, neither simple nor practical. As has been pointed out, the case reported by Wilder et al. still stands alone in completeness of proof of hyperinsulinism and, as Harris ('34) has recently observed, if this criterion is necessary, few diagnoses will be made

before operation or autopsy. The important fact, clinically, is recognition of the condition. Whether the disturbance is due actually to excess production of insulin or abnormal activity of the normal supply because of decreased activity of the thyroid, pituitary, adrenal, or other anti-insulin secretion, is largely of academic interest. Clinically, the signs and symptoms are essentially the same and treatment is the same. Regardless of cause, diet alone suffices; in mild cases, frequent feedings of liberal quantities of carbohydrates usually suffice; in more severe disturbances, high fat diets are, at times, of value, as they tend to cause resistance to insulin. Thyroid medication is of value, and intravenous injection of glucose may be necessary in the more severe cases. When these fail, surgical treatment must be considered (removal of tumor, partial pancreatectomy, etc.). As yet, treatment with anterior pituitary gland is of no value. In rare cases, as with insulin injections, the condition, unless promptly relieved, may be fatal. For this reason, and as there are no means of predicting results, severe disturbances should be treated by prompt use of glucose intravenously.

Classification of hypoglycemia on the basis of etiology is, as yet, not very satisfactory for other reasons. Too little is known, clinically, to attribute hyperinsulinism to the thyroid alone; to the adrenals alone or to other organs of internal secretion. There is, as yet, much to be known about the regulation of blood sugar. The dysinsulinism due to rhythmic activity of the liver with respect to storage and liberation of glycogen is an example of the many difficulties in the interpretation of data (Mollerstrom, '33). Whether this rhythmic activity is controlled by some mechanism in the liver or whether it is due to some extra-hepatic activity, such as alternate stimulation and depression of the insulin-producing mechanism, is, as yet, not known. Classification, according to symptomatology, is further complicated by the finding of symptoms without hypoglycemia; symptoms have been reported when the blood sugar was normal or even above

normal.[2] The latter finding does not fit in with a recent statement by Harris ('33) that hyperinsulinism is invariably associated with hypoglycemia. Our experiences agree with those of Harris. Among diabetics we have not as yet observed insulin reactions without hypoglycemia. Classification is further complicated by the fact that there may be hyperinsulinism without symptoms. In a case reported by Peters and Rabinowitch ('29) there were for a time no symptoms whatever, though the glucose had disappeared from the blood entirely.

As with insulin injections in diabetes, there are a number of conditions which may influence the severity of symptoms. Insulin, as is well known, acts best in young people, in the undernourished, and in the dehydrated individual. Exercise aggravates the symptoms of excess insulin dosage and the disturbance is likely to be severe whenever there is interference with the absorption of food (gastritis, enteritis, etc.). Symptoms tend to be severe in all conditions which tend toward starvation because of depletion of glycogen (diarrhea, vomiting, etc.). Response to insulin is also increased by low fat diets and symptoms may be severe when there is sudden improvement of carbohydrate tolerance. Sudden improvement is common in recovery from infection, injury and a variety of other conditions which tend to cause resistance to injected insulin or to that normally produced in the body (Rabinowitch and Bazin, '24, '28 and '32). Recently, Harris ('33), in a review of the literature, again drew attention to another disturbance of the insulin-producing mechanism, namely, dysinsulinism, a condition in which hyperglycemia may alternate with, or be followed by, hypoglycemia.

Aside from the importance of early diagnosis in severe cases, recognition of hyperinsulinism is necessary because of other possible sequelae. Hyperinsulinism is a nutritional disorder. Harris, for example, suggests that prolonged over-

[2] Under these conditions, the symptoms might be due to a rapidly falling blood sugar alone or possibly to a decrease of sugar in the central nervous system below the critical level without a corresponding decrease in the peripheral blood (Harrison, '26).

function of the islet cells of the pancreas may lead to their exhaustion and thus to diabetes. Hyperinsulinism also tends to cause obesity, another important etiological factor in diabetes. It is of interest here to note that hypoglycemia is, apparently, as common as hyperglycemia (Sippe and Bostock, '33).

METHODS OF INVESTIGATION

Selection of cases. Though not proved anatomically, the subjects investigated were, judging from clinical signs and blood sugar data, suffering from hyperinsulinism. The history in each case was essentially the same, namely, attacks of hunger and weakness which came on after meals, varying between ½ and 3 hours, in spite of what appeared to be the usual and, at times, more than the usual amount of food. In one case, headache accompanied the weakness and hunger. In all cases, the symptoms were completely relieved by taking food. Two of the subjects discovered that eating between meals relieved the symptoms.

EXPERIMENTAL

The experimental procedures were the same as those which have been in use in this laboratory since 1924 (Finley and Rabinowitch, '24). Experiences with hundreds of such tests have increased our confidence in this type of work. They consist of simultaneous determination of blood sugar and respiratory metabolism before and, again, 30, 60, 120 and 150 minutes after administration of glucose. The vagaries of this experimental procedure are many. A variety of precautions are necessary in order to be reasonably certain that the data obtained reflect the metabolic conditions. It may here be observed, it does not necessarily follow that the respiratory quotient at the mouth is the respiratory quotient in the body tissues. There are, first, the many sources of error due to technic. Adjustment of gas masks or mouth pieces; leakage of gas from the bags in which the samples are collected, and errors during gas analyses are the more common sources.

Carefully trained technicians, care of apparatus and long experience with such tests tend to reduce these errors to a minimum. All gas analyses in this investigation were made with the Haldane type of gas apparatus. Accuracy of apparatus was repeatedly checked by analyses of outside air. All analyses were made in duplicate by two technicians. Each gas sample was, therefore, analyzed in quadruplicate. Urinary nitrogen estimations were not made, because of the uncertainty that values obtained at short intervals of time represent the protein metabolism for the corresponding periods. As these subjects were not diabetics—as there was no glycosuria—15 per cent of the total heat production was attributed to protein. For the validity of this procedure, there appear to be sufficient data (Voit, '01). The remaining calories were assumed to be derived from carbohydrate and fat and were apportioned according to the Zuntz-Schumburg tables as modified by Williams, Riche and Lusk ('12). In the calculations of rates of oxidation of carbohydrate, no alterations were made when the respiratory quotients were greater than 1.0 or less than 0.7. In the former case, consumption of oxygen and carbon dioxide production were attributed to oxidation of carbohydrates and, in the latter to oxidation of fat.

Aside from the sources of error due to technic, a variety of other factors must be considered in the interpretation of data. Psychic influences, for example, must be reduced to a minimum. This depends largely upon the personality and training of the technician. Careful attention throughout the test to respiratory movements, to rate and amplitude of respirations, is very important. The influences of the latter will be dealt with in a discussion of the data.

All tests were performed in the morning and under strictly basal metabolism conditions with no alterations whatever in the dietary habits of the individual prior to the day of the test.

In spite of all of the above-mentioned precautions, there remain the possible sources of error inherent in short time experiments. Though the data obtained under these condi-

tions may actually reflect the metabolism at the time of the test, they may not be representative of the conditions during the greater part of the day. Consideration must also be given to a variety of other factors. As these have been dealt with elsewhere (Joslin, '24; Du Bois, '24), they will not be discussed here. Confidence in this procedure is, however, enhanced by the fact that data of some of our previous experiments have been repeatedly confirmed with the aid of the very exact respiration calorimeter at the Russell Sage Institute of Pathology. The latter include the observations of Ladd and Richardson on renal glycosuria ('25) and those of McClellan et al. ('28) on the metabolism of dihydroxyacetone.

The data of five cases are reported in the order in which they were obtained (tables 2 to 6). In all, six cases were studied. One case is not included in this report, because the blood sugar time curve obtained during the test was not flat; the hyperglycemic response to the ingested glucose was normal. In this investigation, for a nmber of reasons to be dealt with later, we were concerned particularly with flat, or nearly flat, curves.

STANDARDS

Blood sugar time curve. The normal blood sugar time curve is too well known to require comment. The conditions necessary to obtain reliable data were dealt with elsewhere by one of the writers (Rabinowitch, '34).

Respiratory exchange. The average respiratory quotient during the fasting period is 0.82 {0.83 for males and 0.81 for females} (Benedict et al., '14). After the administration of 100 gm. of glucose, an increase may be noted within a few minutes (Higgins, '16) and rarely fails to occur within 30 minutes. The average maximum increase is about 0.12. In some instances a slight decrease may be noted. In conditions which deplete glycogen, such as exercise, starvation, etc., no increase or an actual decrease may be noted for a time. Benedict and Carpenter ('18) found a fairly consistent picture in the ten cases observed. The lowest, highest, and average

peaks of carbon dioxide production were, respectively, 12, 35, and 25 per cent above the basal period; of oxygen consumption, 3, 22, and 12 per cent; of the heat production, 6, 24, and 14 per cent; and of the respiratory quotients, 0.07, 0.17 and 0.12, respectively. The average time of appearance of the maximum effect was within 2 hours.

DISCUSSION OF RESULTS

The blood sugar time curves obtained in the five cases in which respiratory metabolism studies were made are shown in table 1. It will be observed that the results, in each case,

TABLE 1

Showing repeated blood sugar time curves in hyperinsulinism

	7727/33		98/34		1000/34		M. S.		295/34		
	Feb. 16	Feb. 21	Jan. 5	Feb. 26	Feb. 23	Mar. 2	Mar. 15	Mar. 19	Jan. 30	Feb. 7	Feb. 14
Before glucose	0.085	0.093	0.088	0.090	0.100	0.098	0.102	0.104	0.108	0.087	0.116
30 minutes later	0.129	0.112	0.120	0.096	0.122	0.131	0.100	0.123	0.126	0.104	0.128
60 minutes later	0.119	0.137	0.102	0.098	0.129	0.128	0.100	0.123	0.125	0.102	0.135
120 minutes later	0.087	0.097	0.095	0.098	0.091	0.100	0.082	0.089	0.122	0.100	0.125
150 minutes later	0.092	0.087	0.083	0.095	0.080	0.100	0.098	0.083	0.111	0.092	0.117

were essentially the same when the tests were repeated. The time interval between tests ranged from 4 days (subject MS) to about 8 weeks (subject 98–34). In one case (subject 295–34) the test was performed three times with a time interval of 8 days between the first and second test and 7 days between the second and third test. These curves, incidentally, demonstrate the value of the test in the detection of hyperinsulinism. As Wilder ('33) has observed, blood sugar time curves obtained singly may not be very helpful. Repeated tests, however, are helpful, when both curves are flat. The value of

repeated tests is, however, destroyed when any changes are made in the dietary habits of the individual between tests (Rabinowitch, '34). The following case of hyperinsulinism (subject JBM) is an example of the disturbing effects of diet. In this case, three blood sugar time curves were obtained with the following results:

	Blood sugar (per cent)		
	Feb. 14	Feb. 26	Mar. 14
Fasting	0.111	0.082	0.112
30 minutes after glucose ingestion	0.116	0.100	0.166
60 minutes after glucose ingestion	0.139	0.122	0.149
120 minutes after glucose ingestion	0.105	0.091	0.128
150 minutes after glucose ingestion	0.103	0.075	0.106

It will be observed that the time interval between the first and second tests was 12 days and between the second and third tests it was 16 days. The first two curves are practically flat; whereas, in the third curve (March 14) the hyperglycemic response to the ingested glucose is normal. Between the second and third tests, the diet was increased not only with respect to carbohydrates, but, also, to caloric content.

In spite of all precautions, flat curves, even when obtained repeatedly, need not necessarily be due to hyperinsulinism; they may still be due to some unrecognizable defect in the absorption of glucose from the alimentary tract. As will presently be shown, however, interpretation of blood sugar data may be simplified by simultaneous study of the effects of the ingested glucose upon the respiratory metabolism. McClelland and Wardlaw ('32) first reported respiratory metabolism studies in hyperinsulinism. As far as could be ascertained from the literature, however, the following present study is the first in which blood sugar and respiratory metabolism data were obtained simultaneously.

Case 1. It will be observed (table 2) that the blood sugar time curve was flat; there was no hyperglycemic response to the ingested glucose. This could have been due to defective absorption of the glucose from the gastro-intestinal tract or to rapid disappearance of the absorbed glucose from the blood

TABLE 2

Hospital no. 98/34. Female. Age 21 years. Height 168.5 cm. Weight = 60 kg. Body surface = 1.67 sq.M. B.M.R. = minus 14 per cent

PERIOD	GASEOUS EXCHANGE				URINARY NITROGEN (GRAMS PER HOUR) CALCULATED [1]	RESPIRATORY QUOTIENT		HEAT PRODUCTION		VENTILATION RATE		RESPIRATION RATE		HEAT PRODUCTION PER HOUR CALORIES DUE TO			FOOD OXIDIZED (GRAMS PER HOUR)			BLOOD SUGAR (PER CENT)
	Carbon dioxide production		Oxygen consumption																	
	Liters per hour	Per cent increase above basal	Liters per hour	Per cent increase above basal		Total	Non-protein	Calories per hour	Per cent increase above basal	Liters per minute	Per cent increase above basal	Respirations per minute	Per cent increase above basal	COH	Fat	Protein	COH	Fat	Protein	
Before glucose	8.90	...	10.90	...	0.297	0.817	0.818	52.04	...	4.309	..	12	..	17.13	27.02	7.89	4.28	2.97	1.92	0.090
30 minutes after glucose	9.08	2.0	11.61	6.4	0.313	0.782	0.777	54.93	5.6	3.987	..	11	..	11.75	34.87	8.32	2.94	3.83	2.03	0.096
60 minutes after glucose	10.09	13.4	12.50	14.7	0.340	0.806	0.807	59.51	14.4	4.410	2.3	11	..	17.87	32.61	9.02	4.47	3.58	2.20	0.098
120 minutes after glucose	9.63	8.2	11.36	4.2	0.312	0.848	0.856	54.70	5.1	4.308	..	11	..	24.32	22.09	8.29	6.08	2.43	2.02	0.098
150 minutes after glucose	9.35	5.1	10.94	0.4	0.301	0.855	0.864	52.63	1.1	4.298	..	11	..	23.40	21.26	7.98	5.85	2.34	1.95	0.095

[1] Assumed to account for 15 per cent of total heat production.

stream, either because of rapid oxidation or storage. It will be noted that the respiratory quotient at the 30-minute period was depressed. This, according to our previous experiences with such tests (Rabinowitch, '25), suggested storage of the ingested glucose. The increase of heat production which was quite appreciable at the 60-minute period is, however, difficult to interpret. It was apparently not due to carbohydrate oxidation and can hardly have been due to stimulation of peristalsis by the ingested fluid. It is a well-known fact that the effects of cathartics and agar-agar are almost negligible, in spite of the intestinal activity which they produce. Without an increased elimination of carbon dioxide (washing out phenomenon), it is difficult to attribute the increase of heat production to excess activity of the respiratory muscles. Oxidation of food stuffs, other than carbohydrate, is the most likely explanation of the increase of heat production.

The respiratory quotient at the end of 120 minutes was increased and, because of the precautions taken, this finding is not regarded as an artefact due to washing out of carbon dioxide. It will be noted that the ventilation and respiration rates also fail to suggest over-ventilation; they were remarkably uniform throughout the test and practically identical with conditions in the fasting (control) period. The cooperation of the patient was ideal. The heat production at this period was increased, but the increase was lower than that which corresponds to the usual specific dynamic action of glucose. It is, however, difficult to attribute it to some other condition. There was no obvious excess activity of the muscles of respiration, nor were there any other abnormal movements. The time factor tends to exclude effects of water ingestion.

In view of the inappreciable increase of heat production at the 150-minute period, the increase of the respiratory quotient is attributed to storage of the ingested glucose, possibly by conversion into fat. Here, also, the ventilation and respiration rate data appear to exclude an artefact due to washing out of carbon dioxide. On the whole, therefore, it would appear that, in this case of suspected hyperinsulinism, the gen-

eral response to the ingested glucose was storage. Since we believe that all recognizable technical and other errors have been excluded, this case affords an example of the value of respiratory metabolism studies when the blood sugar time curve is flat. In this case, defective absorption of the ingested glucose from the alimentary tract is excluded. That our interpretation of the above data is correct is suggested from the experiences with the following case.

Case 2. In this case (table 3) the blood sugar time curve was practically flat; but, differing from the previous case, it will be noted that, following ingestion of the glucose, there was an immediate and sustained increase of the respiratory quotient; and each increase was accompanied by an increase of heat production. Because of the ventilation and respiration rate data, we believe that artefacts due to washing out of carbon dioxide are excluded. It is of interest to note that the percentage increase of carbon dioxide elimination was appreciably greater than the percentage increase of ventilation rate; and that the latter bore little or no relationship to the percentage increase of heat production. In this case of suspected hyperinsulinism, therefore, the dominant response to the ingested glucose was oxidation.

Proof that the flat blood sugar time curve in this case was not due to defective absorption of glucose is much more definite than in the previous case.

Assuming these two individuals were suffering from hyperinsulinism, it would appear that the response to glucose ingestion in this condition is not uniform. Further suggestive are the findings when consideration is given to the dietary habits of these subjects; the data fit in with the generally accepted views of carbohydrate oxidation in animals and with findings in man. In simultaneously determined arterial and venous blood sugar and respiratory quotient time curves in diabetics following insulin administration, Rabinowitch and Bazin ('27) showed that, as in animals, glycogen formation appears to be a necessary preliminary to oxidation of carbohydrates. This offers an explanation of the data in the above-

TABLE 3

Subject M.S. Female Age 26 years. Height 167.6 cm. Weight = 60.4 kg. Body surface = 1.67 sq.M. B.M.R. = minus 5 per cent

PERIOD	GASEOUS EXCHANGE				URINARY NITROGEN (GRAMS PER HOUR) CALCULATED [1]	RESPIRATORY QUOTIENT		HEAT PRODUCTION		VENTILATION RATE		RESPIRATION RATE		HEAT PRODUCTION PER HOUR CALORIES DUE TO			FOOD OXIDIZED (GRAMS PER HOUR)			BLOOD SUGAR (PER CENT)
	Carbon dioxide production		Oxygen consumption																	
	Liters per hour	Per cent increase above basal	Liters per hour	Per cent increase above basal		Total	Non-protein	Calories per hour	Per cent increase above basal	Liters per minute	Per cent increase above basal	Respirations per minute	Per cent increase above basal	COH	Fat	Protein	COH	Fat	Protein	
Before glucose	10.02	...	12.14	..	0.331	0.825	0.829	58.04	...	4.98	...	12	..	20.78	28.47	8.79	5.20	3.13	2.14	0.104
30 minutes after glucose	11.10	10.8	13.08	7.7	0.359	0.849	0.856	62.97	8.5	4.96	...	11	..	28.00	25.43	9.54	7.00	2.79	2.33	0.123
60 minutes after glucose	11.34	13.2	12.68	4.4	0.352	0.895	0.911	61.66	6.2	5.63	13.0	11	..	36.31	16.01	9.34	9.08	1.76	2.28	0.123
120 minutes after glucose	11.99	19.7	12.64	4.1	0.356	0.949	0.977	62.38	7.5	5.44	9.2	10	..	49.33	3.60	9.45	12.33	0.39	2.30	0.089
150 minutes after glucose	11.42	14.0	12.47	2.7	0.349	0.916	0.937	61.02	5.1	5.04	1.2	10	..	41.21	10.56	9.25	10.30	1.16	2.26	0.083

[1] Assumed to account for 15 per cent of total heat production.

mentioned cases of hyperinsulinism. It would thus appear that the response to glucose in hyperinsulinism depends upon the amount of glycogen stored in the body at the time of the test. With a good supply of glycogen, the response to glucose ingestion is oxidation and when there is a tendency toward depletion of glycogen, the initial reaction is storage. The findings in these two cases fit in with the respiratory metabolism data obtained by McClellan and Wardlaw ('32) in another case of hyperinsulinism. They also fit in with the following cases in which the dietary habits of the subject were carefully correlated with the metabolic data. The importance of a knowledge of the dietary habits, in the interpretation of respiratory metabolism data, was emphasized by one of the writers in 1925 in somewhat similar studies (Rabinowitch, '25). In the above-mentioned paper, McClellan and Wardlaw again draw attention to this fact.

Case 3. There are a number of difficulties in the interpretation of the data in this case. Here, also, it will be noted (table 4) that, as in case 2, the blood sugar time curve was practically flat and that the respiratory quotients were increased throughout the test. There are, however, two important differences between the two cases; the respiratory quotient was very low in the fasting state (lower than the respiratory quotient for fat) and it was very high (higher than the respiratory quotient for carbohydrate) at the 120-minute period.

Very low respiratory quotients, less than 0.7, are not very common. They may be met with in the very severe diabetic when the D:N ratio is very high. The late Prof. Graham Lusk ('28) calculated that the diabetic respiratory quotient for protein is 0.632 when the D:N ratio is 3.65. Respiratory quotients lower than 0.7 have also been noted occasionally in normal individuals. Lyon, Dunlop and Stewart ('32) have recently reviewed the literature and drew attention to the low values which may be met with in obesity. These authors suggested the possibility of conversion of fat into sugar. Space does not permit a discussion of this highly controversial

TABLE 4

Hospital no. 1000/34. Male. Age 29 years. Height 180.3 cm. Weight = 61.2 kg. Body surface = 1.77 sq.M. B.M.R. = plus 9 per cent

PERIOD	GASEOUS EXCHANGE: Carbon dioxide production		GASEOUS EXCHANGE: Oxygen consumption		URINARY NITROGEN (GRAMS PER HOUR) CALCULATED [1]	RESPIRATORY QUOTIENT		HEAT PRODUCTION		VENTILATION RATE		RESPIRATION RATE		HEAT PRODUCTION PER HOUR CALORIES DUE TO			FOOD OXIDISED (GRAMS PER HOUR)			BLOOD SUGAR (PER CENT)
	Liters per hour	Per cent increase above basal	Liters per hour	Per cent increase above basal		Total	Non-protein	Calories per hour	Per cent increase above basal	Liters per minute	Per cent increase above basal	Respirations per minute	Per cent increase above basal	COH	Fat	Protein	COH	Fat	Protein	
Before glucose	10.93	...	16.36	..	0.433	0.668	0.642	76.17	..	5.584	...	13	..	....	64.67	11.50	....	7.11	2.80	0.098
30 minutes after glucose	12.45	13.9	17.29	6.3	0.460	0.720	0.704	80.47	5.6	5.964	6.8	13	..	....	68.27	12.19	....	7.50	2.97	0.131
60 minutes after glucose	13.06	19.5	15.03	..	0.416	0.869	0.881	72.61	..	5.923	6.1	13	..	36.46	25.13	11.02	9.12	2.76	2.69	0.128
120 minutes after glucose	13.24	21.1	12.77	..	0.365	1.037	1.083	63.22	..	6.203	11.1	13	..	53.55	....	9.67	13.39	...	2.36	0.100
150 minutes after glucose	13.01	19.0	13.42	..	0.380	0.970	1.001	66.51	..	6.163	10.4	13	..	56.42	....	10.08	14.11	...	2.46	0.100

[1] Assumed to account for 15 per cent of total heat production.

subject. It is, however, an interesting speculation as to whether, in spontaneous hyperinsulinism, when the body is depleted of its carbohydrates, it attempts to obtain sugar from unusual sources. Another explanation, and which perhaps fits in better with established facts of physiology, is that the low respiratory quotient in hyperinsulinism is due to the protein metabolism. It is suggested that conditions in hyperinsulinism may be, at times, somewhat comparable with those in the very severe diabetic with a high D:N ratio; in the diabetic, the low respiratory quotient is due to the fact that the glucose formed from protein is excreted; in spontaneous hyperinsulinism, when there is a tendency toward depletion of glycogen, the glucose so formed is stored.

That the very low respiratory quotient in this case was due to storage of glucose from protein is suggested from the findings after administration of glucose. It will be observed, as stated, that the respiratory quotient was greater than 1.0 at the 120-minute period. An artificially high respiratory quotient due to over-ventilation is, we believe, excluded. There was no obvious over-ventilation throughout the test and it will be noted that there was no relationship between percentage increase of ventilation rate and percentage increase of carbon dioxide elimination.

Further suggestive of storage of the ingested glucose is the fact that, with one exception only (the 30-minute period), increase of respiratory quotient was not accompanied by increase of heat production. (It should also be observed that the respiration rates were remarkably regular and slow.)

The fact that the data show an increased rate of oxidation of carbohydrates (grams carbohydrate oxidized per hour) is not, in our opinion, incompatible with the above interpretation. The values recorded are obviously due to the arbitrary method of calculating rate of oxidation of carbohydrate. It is, of course, possible that glucose may be oxidized without exerting a specific dynamic action. This peculiar phenomenon has been observed before. Coleman and Du Bois ('15), for example, found no appreciable increase of heat production

in typhoid fever during the febrile stage of the disease. This, as Lusk suggested ('14), may have been due to the increase of metabolism. These data are, however, hardly comparable with those obtained in the above case of hyperinsulinism, since there was neither fever nor an increase of basal metabolism. Storage of the ingested glucose is the most likely explanation of our data. Storage, as a matter of fact, is probably the explanation of the absence of specific dynamic action of food in fevers. As Lusk pointed out in his discussion of the protein metabolism in typhoid fever, the protein in fever can never be reduced to as low a level as in the normal individual. Therefore, the protein ingested may serve to replace the protein which is being broken down in excess. Under these conditions, it would not increase the heat production. That the interpretation of our data in the above case of hyperinsulinism is correct is suggested from experiences with the following case:

Case 4. The history of this case is of interest. The patient complained of marked weakness on awakening in the morning; of drowsiness in the afternoon; and of a loss of 20 pounds of weight during the last year. Loss of weight fits in with starvation and, therefore, depletion of body glycogen. Assuming that glycogen formation is a necessary preliminary to oxidation, one could, a priori, expect the first effect of glucose ingestion in this case to be storage. One might also expect, as in case 3, a very low respiratory quotient in the fasting state. It will be noted, however, that the respiratory quotient was not only not low, but much higher than normal (0.907) and it is difficult to attribute it to over-ventilation. Firstly, there was no increase of respiration rate; the respirations were 20 per minute. Secondly, since the ventilation rate was 6.20 liters and the respiration rate was 20 per minute, the average exchange of air was only 310 cc. per respiration and, lastly, the basal metabolic rate was low, namely, minus 9 per cent. This is opposed to excess activity of the muscles of respiration. The high respiratory quotient in the fasting state suggests that, in this case of hyperinsulinism, the body, in

TABLE 5

Hospital no. 7727/33. Male. Age 20 years. Height 167.6 cm. Weight = 59.2 kg. Body surface = 1.66 sq.M. B.M.R. = minus 9 per cent

PERIOD	GASEOUS EXCHANGE: Carbon dioxide production: Liters per hour	GASEOUS EXCHANGE: Carbon dioxide production: Per cent increase above basal	GASEOUS EXCHANGE: Oxygen consumption: Liters per hour	GASEOUS EXCHANGE: Oxygen consumption: Per cent increase above basal	URINARY NITROGEN (GRAMS PER HOUR) CALCULATED [1]	RESPIRATORY QUOTIENT: Total	RESPIRATORY QUOTIENT: Non-protein	HEAT PRODUCTION: Calories per hour	HEAT PRODUCTION: Per cent increase above basal	VENTILATION RATE: Liters per minute	VENTILATION RATE: Per cent increase above basal	RESPIRATION RATE: Respirations per minute	RESPIRATION RATE: Per cent increase above basal	HEAT PRODUCTION PER HOUR CALORIES DUE TO: COH	HEAT PRODUCTION PER HOUR CALORIES DUE TO: Fat	HEAT PRODUCTION PER HOUR CALORIES DUE TO: Protein	FOOD OXIDIZED (GRAMS PER HOUR): COH	FOOD OXIDIZED (GRAMS PER HOUR): Fat	FOOD OXIDIZED (GRAMS PER HOUR): Protein	BLOOD SUGAR (PER CENT)
Before glucose	11.46	...	12.63	...	0.353	0.907	0.927	61.63	...	6.20	...	20	..	39.84	12.44	9.35	9.96	1.37	2.280	0.093
30 minutes after glucose	13.20	15.2	13.68	8.3	0.387	0.965	0.997	67.74	9.9	6.92	11.6	19	..	57.48	....	10.25	14.37	...	2.50	0.112
60 minutes after glucose	13.60	18.7	14.24	12.7	0.402	0.955	0.984	70.26	14.0	6.88	11.0	20	..	55.56	4.05	10.65	13.89	0.44	2.60	0.137
120 minutes after glucose	13.62	18.8	13.95	10.4	0.396	0.977	1.011	69.10	12.1	6.77	9.2	20	..	58.60	....	10.51	14.65	...	2.56	0.097
150 minutes after glucose	12.80	11.7	11.24	...	0.321	1.139	1.207	55.65	...	7.31	19.5	19	..	47.14	....	8.51	11.78	...	2.075	0.087

[1] Assumed to account for 15 per cent of total heat production.

spite of loss of weight, had a good supply of glycogen, a supply which permitted continuous oxidation of appreciable quantities of carbohydrate even as late as 15 hours after the last meal. That this is the correct explanation is suggested from the history of the case. This patient learned by experiment that the weakness and hunger which came on regularly about 2 hours after meals could be relieved by eating between meals. He has, therefore, followed this practice for some time. This offers an explanation of the findings after administration of the glucose. It will be observed that, differing from the previous case, the prominent response to the ingested glucose was oxidation. Proof of this is that, with one exception, each increase of respiratory quotient was accompanied by an increase of heat production which corresponded to the normal specific dynamic action of glucose. That this increase of heat production was due to the specific dynamic action of glucose and not to over-ventilation is again suggested from correlation of ventilation rate with carbon dioxide elimination and heat production.

There is one finding difficult to interpret in this case. It will be observed that the very high respiratory quotient at the 150-minute period was not accompanied by an increase of heat production. The respiratory quotient, being greater than unity, suggests conversion of glucose to fat. The increase of ventilation rate suggests an artefact and, according to the clinical history, it would appear that storage of ingested glucose was not necessary. On the other hand, there was not only no increase of heat production, but actually a decrease. This suggests storage.

Case 5. In this case (table 6) some of the data are also difficult to interpret. It would appear that there was a combination of storage and oxidation. As in the previous case, the respiratory quotient is high in the fasting state; and, here, also, the data fit in with the history of the case. This man suffered from diarrhea and loss of weight before the test and these were attributed to a chronic pancreatitis. A high carbohydrate-low fat diet was, therefore, substituted for the

TABLE 6

Hospital no. 295/34. Male. Age 43 years. Height 160.2 cm. Weight = 51.36 kg. Body surface = 1.51 sq.M. B.M.E. = plus 7 per cent

PERIOD	GASEOUS EXCHANGE				URINARY NITROGEN (GRAMS PER HOUR) CALCULATED [1]	RESPIRATORY QUOTIENT		HEAT PRODUCTION		VENTILATION RATE		RESPIRATION RATE		HEAT PRODUCTION PER HOUR CALORIES DUE TO			FOOD OXIDIZED (GRAMS PER HOUR)			BLOOD SUGAR (PER CENT)
	Carbon dioxide production		Oxygen consumption																	
	Liters per hour	Per cent increase above basal	Liters per hour	Per cent increase above basal		Total	Non-protein	Calories per hour	Per cent increase above basal	Liters per minute	Per cent increase above basal	Respirations per minute	Per cent increase above basal	COH	Fat	Protein	COH	Fat	Protein	
Before glucose	11.17	...	12.57	..	0.349	0.889	0.904	61.01	...	6.790	...	..	..	34.16	17.59	9.261	8.539	1.933	2.259	0.116
30 minutes after glucose	14.15	26.7	12.58	0.1	0.359	1.124	1.189	62.32	2.1	7.752	14.1	..	..	52.79	....	9.524	13.198	....	2.323	0.128
60 minutes after glucose	14.09	26.1	12.79	1.7	0.365	1.101	1.161	63.33	3.8	7.216	6.3	..	..	53.65	....	9.683	13.41	....	2.362	0.135
120 minutes after glucose	14.86	33.0	13.70	9.0	0.391	1.085	1.141	67.86	11.2	7.665	12.8	..	..	57.48	....	10.37	14.37	....	2.530	0.125
150 minutes after glucose	14.24	27.5	12.99	3.3	0.371	1.096	1.155	64.34	5.4	7.254	6.8	..	..	54.51	....	9.834	13.63	....	2.398	0.117

[1] Assumed to account for 15 per cent of total heat production.

normal diet 11 days before the test and was continued until 3 days before the test when he was given a full hospital diet. He was also given frequent feedings. Since protein must have contributed appreciably to the heat production during the loss of weight, it would appear that the data are somewhat comparable to those which were obtained in typhoid fever by Du Bois when a high carbohydrate was substituted for a high fat diet. It will be noted that all of the respiratory quotients after administration of the glucose were greater than 1.0 and, that except for the 120-minute period, the increase of heat production was below the minimum specific dynamic action of glucose in normal individuals (Benedict and Carpenter, '18). Over-ventilation does not appear to account for these very high respiratory quotients, according to percentage increase of carbon dioxide elimination, ventilation rate and heat production.

The basal metabolic rate was also normal, namely, plus 7 per cent. The above data are, therefore, attributed to a combination of oxidation and storage. It is suggested that, because of loss of weight due to the chronic diarrhea, there was a tendency to store the ingested glucose as fat, in order to restore body weight. This would account for the respiratory quotients greater than unity. As the patient received a liberal carbohydrate diet for some days before the test, this restored the glycogen in the body. (There was no acetone in the urine.) With a good supply of glycogen, some of the glucose ingested was oxidized.

It was considered of interest to examine the respiratory metabolism data for any harmful effects of hyperinsulinism other than those due to hypoglycemic disturbances. As previously stated, Harris suggested that prolonged over-function of the cells of the pancreas may lead to their exhaustion and thus to diabetes. In our cases, at least to date, there is no evidence of such exhaustion. It will be noted that the blood sugar time curves in all of the above cases were flat or nearly so. Flat blood sugar time curves, when not due to defective absorption of sugar from the gastro-intestinal tract, suggest exceptionally good carbohydrate tolerance.

That exposure to excess insulin may eventually disturb sugar tolerance and need not necessarily do so by exhaustion of the islet cells of the pancreas is suggested from our experiences in 1927,[3] with repeated blood sugar time curves obtained in a normal individual following administration of insulin. The following is an example:

In this case (Hosp. no. 5848/27) the patient was suffering from an eczema and the blood sugar time curve suggested a slight disturbance of carbohydrate tolerance. It was considered of interest to test the effects of insulin upon the course of the eczema. The following are the results of six blood sugar time curves obtained during the period of observation:

	Date (blood sugar—per cent)					
	Mar. 7	Oct. 6	Nov. 5	Nov. 12	Nov. 17	Nov. 23
Fasting	0.106	0.093	0.084	0.114	0.120	0.099
30 minutes after ingestion	0.149	0.175	0.222	0.250	0.232	0.166
60 minutes after ingestion	0.196	0.204	0.250	0.385	0.454	0.250
120 minutes after ingestion	0.101	0.111	0.196	0.416	0.357	0.476
150 minutes after ingestion	0.109	0.106	0.075	0.238	0.181	0.250

The first two blood sugar time curves (Mar. 7 and Oct. 6) were obtained before insulin. The third curve (Nov. 5) was obtained 3 weeks after insulin treatment was instituted. It will be noted that there was a marked disturbance of the carbohydrate metabolism; and according to the subsequent curves the disturbance appeared to be progressive. All six curves were obtained under the strict conditions previously described (Rabinowitch, '34). Errors are, therefore, excluded. This patient was kept in the hospital from October 5th to November 24th and no alterations were made in the diet between tests. In spite of repeated physical examinations, other than the exzema, nothing was found to account for the progressive disturbance of carbohydrate tolerance. Unfortunately, the patient refused further treatment. He was discharged from the hospital and has not been heard of since.

That the relationship noted between the insulin treatment and disturbance of carbohydrate tolerance was causal and not

[3] Unpublished data.

accidental is suggested from somewhat similar experiences in other cases. Interpretation of the findings is, however, difficult. Experimental data with respect to the effects of insulin on normal animals are conflicting. Some authors have found that insulin and sugar administered simultaneously caused an increased storage of glycogen in the body; the insulin so administered produced the same physiological effects as in the diabetic, but with more intensity. Others have failed to observe such effects; not only was there no increase of utilization of carbohydrates, but large quantities of insulin were found to suppress glycogen formation almost completely. In some cases, the course of the ingested glucose could not be traced. The whole subject was excellently reviewed recently by Cori ('31). The findings of Chaikoff and Macleod ('27) are of special interest here. When normal rabbits were fed ample carbohydrates, insulin did not increase carbohydrate oxidation and, from the data available at the time, Macleod ('26) concluded that the difference between the normal and diabetic animal is due to the fact that the normal animal can at all times release from the pancreas a sufficient supply of insulin to metabolize or polymerize whatever amounts of carbohydrate may be present in the body. In other words, the endogenous supply of insulin is always at an optimum in the normal animal.

There are very few respiratory metabolism data showing the effects of simultaneous administration of glucose and insulin in normal human individuals. From the data obtained by Rabinowitch and Bazin ('28) it would appear that, unlike in diabetes, insulin does not increase the rate of oxidation of administered carbohydrates. From data then available, animal and human, it was suggested that insulin does not only not enhance oxidation of sugar in the normal individual, but in some as yet unexplained way interferes with the normal mechanism. The data fit in with more recent findings of other workers. Wilder, Smith and Sandiford ('32), in a study of obesity, noted that administration of insulin for periods of from 12 to 30 days was followed by temporary loss of carbo-

hydrate tolerance to such an extent that the blood sugar time curves were typical of diabetes mellitus. Paul, Clark and Gibson ('32) studied the effects of insulin in a non-diabetic who suffered from anorexia. Hyperglycemia and glycosuria developed when the insulin was discontinued. In a study of five other non-diabetics who were given insulin three times a day in relationship to meals, the results were essentially the same and blood sugar time curves in three of these cases indicated diminished sugar tolerance. Data obtained by McJunkin and

TABLE 7

Showing increases of respiratory quotients and percentage increases of oxygen consumption, carbon dioxide production and heat production in normal individuals following administration of glucose with and without insulin and in hyperinsulinism

	RESPIRATORY QUOTIENT			OXYGEN CONSUMPTION			CARBON DIOXIDE PRODUCTION			HEAT PRODUCTION		
	Low	High	Average	Low	High	Average	Low	High	Average	Low	High	Average
Normal[1]	0.07	0.17	0.12	3	22	12	12	35	25	6	24	14
Glucose and insulin administered simultaneously to normal individuals[2]	0.01	0.14	0.06	1	24	9	0	46	13	1	26	10
Hyperinsulinism	0	0.35	0.15	0	14.7	4.8	2	33	16.9	0	14.4	5.9

[1] Benedict and Carpenter—Carnegie Inst. Wash. Pub. no. 261.

[2] Rabinowitch and Bazin—J. Biol. Chem., vol. 80, p. 723, '28.

Roberts ('32) are of interest here. In a study of the effects of excessive insulin on the pancreatic islet cells in young rats, these workers noted that the injected insulin inhibited the proliferative activity of the islet cells, though the rate of growth of the animals otherwise was not impaired. More recent experiences, therefore, also suggest that injected insulin not only does not enhance oxidation of sugar in normal individuals, but interferes with the action of the insulin produced by the body. Does this also apply to the excess insulin to which the body is exposed in hyperinsulinism? The data in table 7 are of interest here. In this table are summarized

the findings of the above five cases of spontaneous hyperinsulinism. These are compared with the results obtained by simultaneous administration of glucose and insulin in normal individuals (Rabinowitch and Bazin, '28) and with the data obtained by Benedict and Carpenter with glucose alone (Benedict and Carpenter, '18). It will be observed that the average values in the cases of hyperinsulinism paralleled more closely those obtained with simultaneously administered insulin and glucose in normal individuals than those obtained with glucose alone. It would, therefore, appear that hyperinsulinism may, at times, not only not cause an increase, but, in some as yet unknown manner, may tend to depress oxidation of carbohydrates.

SUMMARY

Simultaneous blood sugar and respiratory metabolism time curves were obtained in five cases of hyperinsulinism after oral administration of glucose.

In each case, the diagnosis of hyperinsulinism was based upon the history and blood sugar findings; the patients complained of weakness, fatigue, hunger, etc., which appeared within a few hours after meals and were relieved by taking carbohydrate-containing foods; and, in each case, repeated blood sugar time curves obtained after administration of glucose were flat; hyperglycemic response to the ingested glucose was inappreciable or absent.

According to the respiratory metabolism data, the response to glucose ingestion may be oxidation or storage; and it would appear that the type of response depends upon the amount of glycogen in the body. With a poor supply of glycogen, the tendency is toward storage and, when the diet is liberal with respect to carbohydrates, the tendency is toward oxidation.

As in all of these cases the clinical histories and symptoms were essentially the same, it would appear that the symptoms of spontaneous hyperinsulinism are largely independent of the glycogen content of the body and are due essentially to disappearance of sugar from the blood stream. Either oxida-

tion or storage may affect such disappearance. The condition thus resembles insulin shock due to insulin injections.

No proof was found that the rate of oxidation of administered carbohydrates is increased in hyperinsulinism. As a matter of fact, the average values suggested a decrease and corresponded somewhat to those obtained by simultaneously administered glucose and insulin in normal (non-diabetic) individuals.

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HUMAN CALORIMETRY

I. A SEMI-AUTOMATIC RESPIRATION CALORIMETER

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NINE FIGURES

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Since the accurate proof that in a given period the energy output of the animal body is approximately equal to the energy produced by the oxidations occurring in the body in that period, direct calorimetry has taken a position of less general interest. If the energy production may be calculated with confidence from a knowledge of the oxygen consumed, of the urinary nitrogen excreted, and of the respiratory quotient making use of a table such as that of Zuntz and Schumburg ('01), then the measurement of the respiratory exchange suffices for many purposes and requires relatively simple apparatus for its determination. There remains, however, a class of problems that demand for their solution the simultaneous measurement of the respiratory exchange and of the heat output of the body. When processes other than the oxidation of fat, protein and carbohydrate are taking place in the body to any considerable amount, energy and gaseous exchanges of unknown magnitude may be involved and our knowledge of the calorific value of a liter of absorbed oxygen is inadequate. Many such processes are known to be of common occurrence, among them being the conversion of carbohydrate to fat, possibly the reverse conversion of fat to carbohydrate; the change from sugar to glycogen and of glycogen to lactic acid, the influence of various hormones on metabolic processes, etc.

Also in studies of the heat regulating mechanism of the body in normal and disturbed conditions, measurement of the heat elimination as well as of the heat production is, of course, essential. The calorimeter to be described was designed for study of the problems in these fields.

The end in view at its construction was to have a respiration calorimeter of working accuracy, comparatively inexpensive in its initial cost, and as automatic in its operation as possible. The ideal was a machine operable by one man alone. In the measurement of the direct heat, almost complete automaticity has been achieved. The measurement of the respiratory exchange occupies about two-thirds of the time of one man. In routine practice two observers are used, the presence of the second being desirable to see that the automatic features are working properly, to take any additional measurements required in any experiment and to ensure, for the peace of mind of the subject, that the calorimeter is never left unattended.

Certain details of the construction are explained by the fact that it was designed for studies of heat production and heat elimination in fevers produced artificially by diathermy and by the high-frequency condensor field. To this end, glass and other non-conducting materials had to be used instead of metal where the latter perhaps might have been preferred if the accuracy of calorimetry were alone to be considered.

GENERAL PLAN OF MEASUREMENT OF GASEOUS EXCHANGE OF HEAT

Figure 1 is a schematic drawing showing the method of measurement of the respiration calorimeter. For the gaseous exchange the closed-circuit system is used. The air in the air-tight chamber in which the subject lies is forced continuously by the pump through the circuit, which includes a Benedict train of absorbers. Water vapor is absorbed by two bottles of sulphuric acid; carbon dioxide by a soda lime bottle followed by one of sulphuric acid. Two such trains are interchangeable in the circuit by the simultaneous throw of two

valves so that one may be weighed while the other is absorbing. Oxygen is admitted automatically as it is used by the subject. Its amount is measured as it enters by a gas meter.

The portion of the heat that is lost from the chamber by evaporation of water is calculated from the amount of water collecting in the acid bottles. Of the remaining heat, lost by radiation and convection, the major part is absorbed by cold water flowing through a grid of glass pipes in the chamber. Before entering, the water is precooled and then brought to a

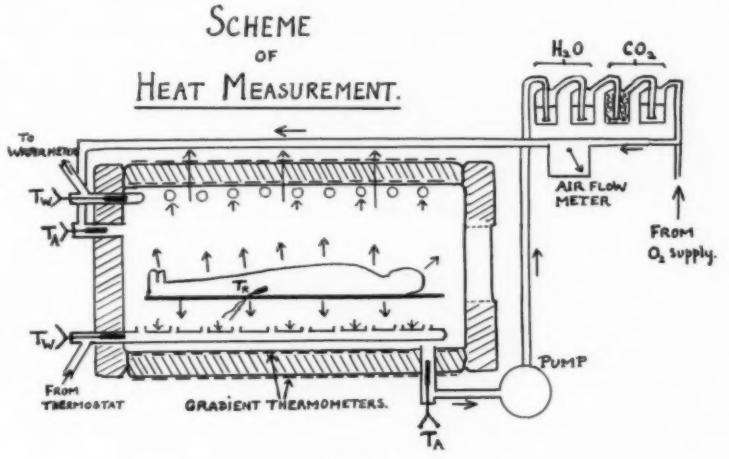

Figure 1

constant temperature in a thermostated bath. The difference of temperature of the water entering and leaving the chamber is measured and automatically recorded by electrical thermometers (T_w in the diagram). The average difference of temperature multiplied by the weight of water flowing in any period, also automatically measured by a water meter of special design gives the number of calories of heat entering the water. A small amount of heat is exchanged with the circulating air. For its calculation the rise of temperature of the air is recorded by the electrical thermometers T_A and the rate of flow of air is read from a simple air flow meter.

The complicated construction of most of the existing calorimeters arises from the attempt to make them adiabatic. A series of enclosing walls are necessary that may be heated or cooled to prevent the escape or ingress of heat from the chamber except by the absorbing system provided. Great simplification in this calorimeter has been achieved by abandoning the adiabatic principle and instead measuring the heat escaping from the calorimeter into its surroundings. It may be noted that in modern purely physical calorimetry, the adiabatic principle is not in general favor, equal and probably greater accuracy being achieved, after the losses of heat have been minimized, by measurement of the losses that remain. Further, no less accuracy of measurement is required to know that the surrounding walls of the calorimeter are at the same temperature than is needed to measure the small difference of temperature that may exist across the insulation.

In the calorimeter here described, heat loss is reduced by the use of an insulating layer surrounding the calorimeter wall, the remaining heat loss being then measured by the thermal gradient set up by the heat flow across the insulation. Electrical thermometers, one inside, one outside the insulating layer, record the gradient of temperature automatically. Calibration has enabled a graph to be drawn giving the heat loss corresponding to any given gradient.

DETAILS OF CONSTRUCTION

The calorimeter room. The room in which the calorimeter is installed measures 17 feet (5M) by 8 feet 6 inches (2.6M) by 10 feet (3M) high, and was built originally for an incubator room. Its walls and doors are lagged with 3 inches (7.5 cm.) of cork insulation. Four electrical heaters at widely spaced points in the room are regulated by a thermostat hanging above the calorimeter. By introducing a series of pipes carrying brine from the refrigerator plant and balancing the heat output of the electric heaters, a lower range of regulation was secured and the room may now be kept to any temperature between 20°C. and 28°C. with a constancy of about $\frac{1}{2}$°C. The calorimeter chamber, with water circulating system and

the air circulating system with the Benedict train, occupy one side of the room. Against the opposite wall are the balance and electrical controls and recording devices (fig. 7).

The chamber. The chamber consists of four cylindrical sections of Pyrex glass, 23 inches (57.5 cm.) in diameter; each section being 19 inches (47.5 cm.) long. The last section is closed at one end. Flanges at the edges of each section enable

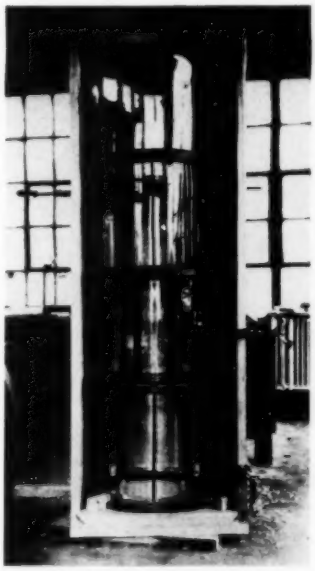

Fig. 2 Glass chamber of calorimeter, showing manner of binding together the segments with bakelite rods and rings.

them to be clamped together with a rubber gasket interposed, by an arrangement of bakelite rings into which bakelite rods are threaded to supply tension and to form a rigid framework round the glass (fig. 2). In addition 'Vulcatex' cement ('knife grade C') is used to seal the joints between the sections. A shorter closed section, 7 inches (17.5 cm.) depth, forms the head of the chamber.[1]

[1] The chamber and mounting were made specially for the calorimeter by the Corning Glass Company, Corning, New York.

The glass chamber is cradled in a hardwood box of external dimensions 29 inches (72.5 cm.) by 29 inches (72.5 cm.) by 82 inches (205 cm.) long. The glass rests upon wooden saddle pieces at each junction of the sections. The last, closed glass section is pressed against the wooden end piece of the box through a rubber pad. A space 1½ inches (3.75 cm.) wide at its narrowest parts exists between the glass cylinder and the wooden walls. It is occupied by the heat insulation to be described later. Several holes for the entrance and exit of the water pipes, the air-flow pipes and of electrical wiring are bored through the glass walls and end piece. A glass window 12 inches (30 cm.) square, in the wooden frame that holds the glass head of the chamber admits light and permits observation of the subject. The enclosing wooden box is supported on two wooden 'horses' so that the axis of the cylindrical chamber is some 2 feet 9 inches (83 cm.) from the floor.

Rapid and effective air-tight closure is made by the arrangement of eight equally spaced steel rods, hinged to the outside of the box so that they swing into engagement with notched metal pieces on the wooden head. Knurled hand-wheels on the threaded rods then clamp the bakelite rings of the head and chamber sections together through a rubber gasket which is cemented permanently to the head. Vaseline is used as a lubricant on the gasket.

The air circulation system. A rotary blower, fitted with a pressure equalizing chamber, is driven at 600 r.p.m. by a 110-volt motor. It is capable of circulating the air through the apparatus at rates up to 60 liters per minute. The rate is controlled by the setting of a tap in a short tube by-passing the air directly from outlet to inlet of the pump. A rate of about 35 liters per minute is usually employed. Brass piping of ½ inch (1.25 cm.) inside diameter leads the air up to the absorbing train on the bench above. The two Benedict trains, each of the four bottles of 2 liters' capacity are mounted in parallel, the air being diverted through one train or the other by the throw of a lever on a rod which turns simultaneously two ground-in metal valves, placed one at each end of the

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Figure 3 shows the simple air flow meter that has been found to be very satisfactory in operation. Brass is used through-

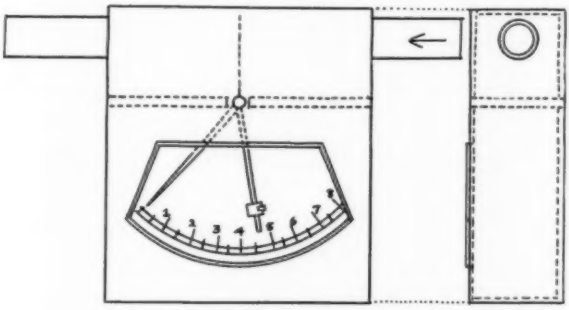

Figure 3

out. A dividing shelf makes a 1-inch (2.5 cm.) square channel, which is almost completely blocked by a light vane (in dotted lines in the figure). The vane is pivoted at the level of the base of the channel and carries a pointer and counterpoise beneath so that under the influence of gravity it stands vertically. Flow of air deflects the vane to a new equilibrium position indicated by the pointer on a scale viewed through a glass window. Adjustment of sensitivity is made by movement of the counterpoise weight on its rod. Calibration, made by collection of the air flowing through the meter in a large spirometer, shows that the scale is practically linear in the range of rates of flow used. The rate in liters per minute

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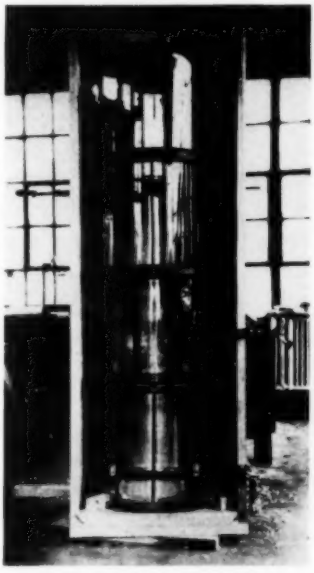

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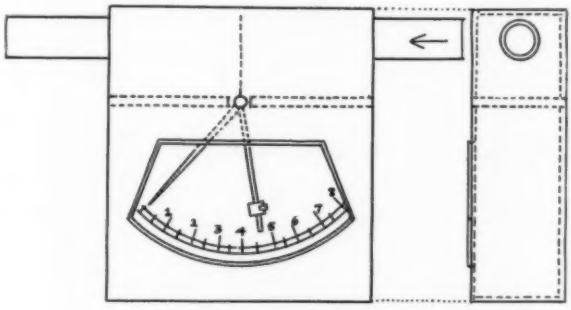

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is read from the calibration curve. Owing to the small magnitude of the specific heat and density of air, the item of heat exchange with the air is in any case a small one, and for its calculation this simple meter is quite accurate enough.

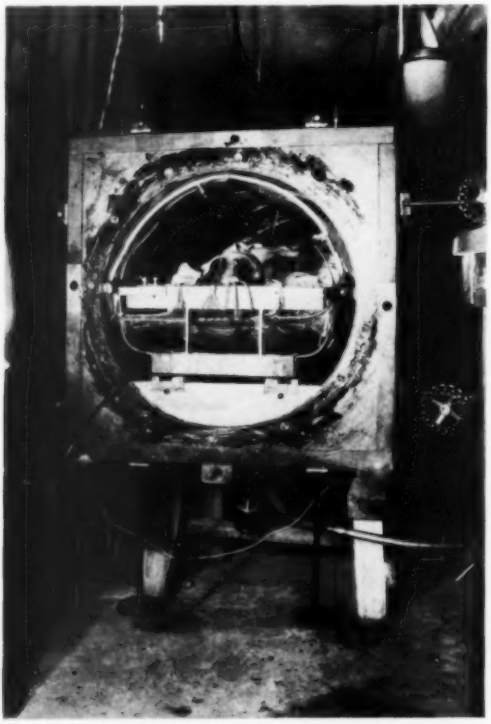

Fig. 4 Chamber open with subject in position. The subject's hand grasps a 'buzzer' button. The arching elements of the glass grid can be seen in the upper part of the chamber.

The oxygen supply. A spirometer of some 4 liters' capacity is connected to the chamber and ensures that the pressure inside is always atmospheric. It may be seen to the right-hand side in the photograph, figure 4. One millimeter movement of the pointer attached to the bell corresponds to

a volume change of 18 cc. When the spirometer bell falls below a certain level, a pin on the counterpoise pulley pushes down an arm making contact with mercury in a cup. This contact actuates a relay, operated on 3 volts, which closes a 110-volt current to energize a magnetically controlled 'pinch cock' admitting oxygen from a cylinder into the system. As the spirometer then rises, a spring keeps the arm in contact until the pin eventually lifts it from the cup, thus breaking the circuit and stopping the inflow of oxygen (Riche and Soderstrom, '15). If for any reason the oxygen supply should fail, the spirometer continues to fall until contact is made with mercury in a second cup, when a bell rings warning the observers that oxygen is needed.

Before it enters the wet-test meter, the oxygen is saturated with water vapor by bubbling through water. As it leaves the meter it is dried by passage through a sulphuric acid tower, and is admitted into the system on the dry side of the Benedict train.

The water circulating system. Distilled water circulates in a closed system constructed wherever possible of block tin. A centrifugal pump forces it up to a tank holding about 27 liters, from which an overflow pipe maintains a constant level and pressure head, which amounts to about 6 feet (2 M.) and suffices to drive up to 2 liters of water per minute through the calorimeter. As it is forced up to the tank, any desired fraction of the water may be diverted through a block tin coil immersed in a bath of ice and water, so that by adjustment of valves the temperature of the water reaching the tank may be regulated to constancy within $\frac{1}{2}$°C. From the tank the water flows through a tin spiral immersed in a stirred 'reheater' bath, which has heating lamps controlled by a sensitive toluene-mercury temperature regulator. The temperature of the water entering the chamber can thus be maintained constant at a desired level, usually between 16 and 17°C., with great accuracy. The constancy of this initial temperature is essential to the accurate measurement of the heat exchange.

The water enters the chamber at the center of the closed glass end, and flows in a Pyrex glass tube parallel to the axis along one side of the chamber. Similar tubes run parallel to this along the top and along the opposite side of the cylindrical chamber. A series of forty Pyrex glass tubes of $\frac{1}{8}$ inch (3 mm.) internal diameter arch across (fig. 4) to connect the three larger tubes like the ribs of a boat. The water which enters the system at the back of the chamber is led back from the front end of the tube lying along the opposite side of the chamber, so that the path through each connecting arch offers equal resistance and there is even distribution of flow. An outlet at the highest point, which is at the open end of the chamber, allows trapped bubbles of air to be released. The total calculated absorbing area is about 10 sq.meters. The grid is painted with lampblack to increase its absorbing power.[2]

The water on leaving the calorimeter flows through an automatic measuring device of special design into the lower tank from which it started. None of the common types of liquid flow meters in commercial use can be relied upon to measure accurately rates of flow as small as 1 or 2 liters per minute, especially at so low a head of pressure. Types of meter which weigh the water have been described, a very ingenius one being described as used in the original Atwater-Rosa-Benedict calorimeter (Atwater and Benedict, '05). The meter used here has fewer moving parts and does not depend upon weighing, but upon volume and so avoids errors due to friction. While of comparatively simplicity, it has proved accurate and dependable as well as completely automatic in operation; for this reason details of its design are here given.[3] The meter is shown in figure 5. The two cylindrical cans of about 4 liters' capacity are mounted vertically side by side. At the bottom of each is an outlet controlled by a conical

[2] The entire grid was made and installed as one piece by William Pfeiffer, 67 Walzford Rd., Rochester, New York.

[3] Much of the design and its successful construction are due to Mr. L. Hofschneider, technician of this laboratory.

rubber valve, normally held close by gravity. One or other of these valves is opened by rocking of the horizontal arm beneath about a pivot at its center. This arm carries rigidly also a vertical piece which serves to swing the pipe carrying the entering water from one can to the other. In the diagram

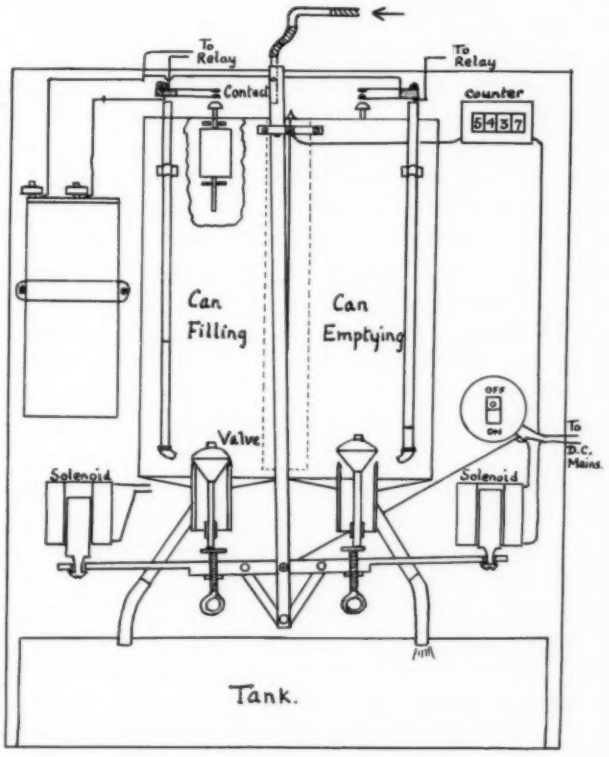

Fig. 5 Recording water meter.

the water is shown filling the left-hand can (observe meniscus in glass gauge), until finally a hollow cylindrical metal float rises to close a pair of light contacts above it. Current from a dry cell will then actuate a relay (not shown), which throws current supplied by the 110-volt mains, from the solenoid on

the right-hand side of the rocker arm to that on the left. The rocker swings, lifting the left-hand valve and discharging that can, and swinging the waterpipe to the empty can, the valve which is now closed. As the vertical arm swings, a contact mounted on it causes a 'telephone' counter to record the number of times the cans have emptied. The relay mentioned has an armature pivoted to lie between two electromagnets, one of which is energized by the closing of the set of contacts above each float. Gravity holds the armature in position against either one or the other electromagnet, in which position an arm attached to the armature carries the 110-volt current through one of a pair of mercury cups to the respective solenoid, until a momentary impulse in the other electromagnet pulls it over to lie against that magnet while the can is filling. Thus, although the closing of the contacts by the floats is momentary only, there is always a positive force supplied by one of the solenoids to hold the mechanism in position. The quantity of water delivered by each can, adjusted by the position of the contacts above the floats to be accurately equal, is found to be constant to within 5 cc. (in a total for each can of 4 liters). For the calculation of the heat, the weight of water flowing is required rather than the volume, but since the temperature of the flowing water does not vary by more than a degree centigrade, the density varies so little that it may be assumed constant.

The bed. The bed is a result of trial and error to obtain the greatest comfort in the limited space. It moves upon casters that roll in brass channelling inserted into wooden rails resting in the chamber. The framework of the bed is of oak, the bed itself being made from a canvas hammock. A wide strip of elastic cloth under the back of the subject is found to give additional comfort. When the chamber is open, the bed may be drawn almost completely out on extension rails carried on a trestle which can be placed temporarily in position. The position of the subject in the chamber is seen in figure 4. There is room for him to turn on his side and to make the small movements necessary to his comfort. Free circulation of air all around the subject is provided.

Communication. Communication with the subject is maintained by an electrical signaling system. The cord and key that is held in the hand of the subject are seen descending from the roof of the chamber in figure 4. A similar key outside the box operates a buzzer mounted beneath the track. A simple code of signals suffices for the experimental routine.

THE MEASUREMENT OF TEMPERATURES

The differential water themometers. At the point of entrance and of exit of the water in the chamber, electrical resistance thermometers are immersed in the stream. These are wound of nickel wire to a resistance of 100 ohms. They are enclosed in brass tubing, some 8 inches of which lie axially in the center of the glass tubing carrying the water, and are connected to form a differential thermometer, in a Wheatstone network, an arrangement too well known to need description here. The current includes a zero adjustment by which the bridge may be balanced accurately when the thermometers are at the same temperature, and a sensitivity adjustment for regulating the readings to different ranges of temperature. Standardization is made by adjustment of the current supplied to the bridge to a standard value shown by a milliameter. The difference of temperature between inflowing and outflowing water is then directly recorded on a Cambridge and Paul thread recording galvanometer, the full scale of which represents 1°C.[4] Calibration has been made by immersion of the thermometers in stirred water baths thermostated in the usual way.

A rectal thermometer of the usual electrical resistance type is also connected to the same recorder so that its resistance is recorded on the same chart on alternate minutes with the water difference. For later work, however, this thermometer has not been of sufficient sensitivity, and another, to be described with those used to measure surface temperatures, has been constructed.

[4] The thermometers, bridge and recorder were supplied especially for the purpose by the Cambridge Instrument Company, of Ossining New York.

The differential air thermometers. The thermometers used to measure the temperature of incoming and outgoing air were originally of a type of construction similar to those used for the water temperatures. It was found, however, that there was a tendency to take up the temperatures of the heavy metal head of the thermometer protruding from the calorimeter box into the room, rather than the temperature of the flowing air. Thermometers of less thermal capacity and thermally insulated from the room were therefore made by winding the resistance coils, covered only by a coating of shellac, on the end of fiber rods pushed axially down the air-pipes at the entrance and exit. The difference of temperature is recorded every minute on a second Cambridge and Paul recording galvanometer. Standardization is here made by the use of a test deflection produced when, for the two resistance thermometers, two fixed resistances differing by a known amount are substituted. A switch is included also by which the resistance of one of the thermometers, the 'exit' thermometer, is compared with a fixed resistance so that the absolute value of the temperature is recorded instead of the temperature difference. This is used, during the taking of 'residuals' at the conclusion of each period, to find the temperature of the chamber air.

The gradient and wall thermometers. In figure 6 the heat insulation is removed from one section of the glass wall of the chamber to show the resistance thermometer that is wound spirally on the outside of the cylindrical wall to give automatically the average temperature over its surface. Nickel wire of no. 40 gauge is attached by sewing to the long strip of cloth shown, there being about 170 feet of wire in all and a total resistance of over 1000 ohms. The insulation consists of a blanket 1½ inches (3.75 cm.) thick, of 'dry zero,' a kapok-like material that has very constant insulating properties. Outside the blanket is a second resistance thermometer in a strip of cloth (shown to the left) spirally covering the cylindrical area. The difference of temperature between these two thermometers gives the thermal gradient to which the escape

of heat through the insulation is proportional. The second recording galvanometer is double and records this gradient every 2 minutes. Alternate minutes an automatic switching device driven by the same clockwork, compares the inner, wall thermometer with a fixed resistance coil, to record the wall temperature. The recording galvanometers are seen on the shelf in figure 7 with the bridges and batteries below.

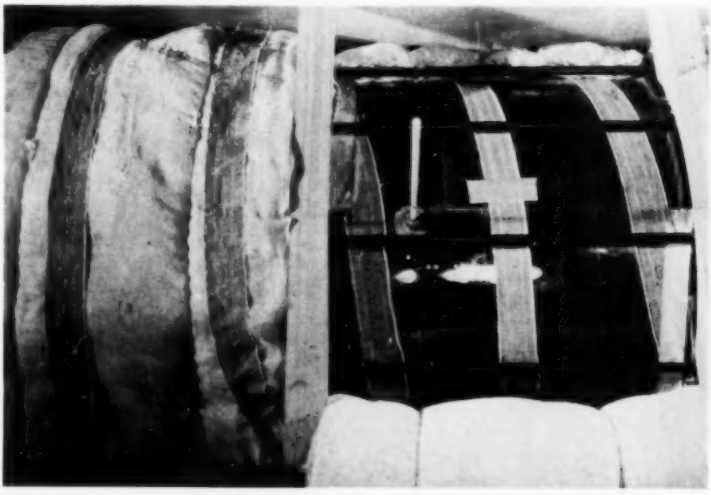

Fig. 6 Insulation of chamber and gradient thermometers.

Rectal and surface thermometers. In order that the calculation of heat stored or lost by the body of the subject may be made as accurately as possible, the average temperatures over areas of the body surfaces have been measured as well as the rectal temperature in all of the later experiments. The technic is that already described by Burton ('34), in which resistance thermometers are made by sewing nickel wire into areas of light gauze cloth which are held close to the skin. One of these thermometers is worn over the surface of the trunk, one over the lower leg and one over the forearm of the subject, the average temperature for other parts of the

surface being estimated by interpolation from these. Clothing may be worn over the gauze thermometers, so that conditions are as normal as possible. The rectal thermometer is wound of the same no. 40 gauge nickel wire on the end of a hard rubber rod of ¼ inch (0.62 cm.) diameter. A spherical swelling

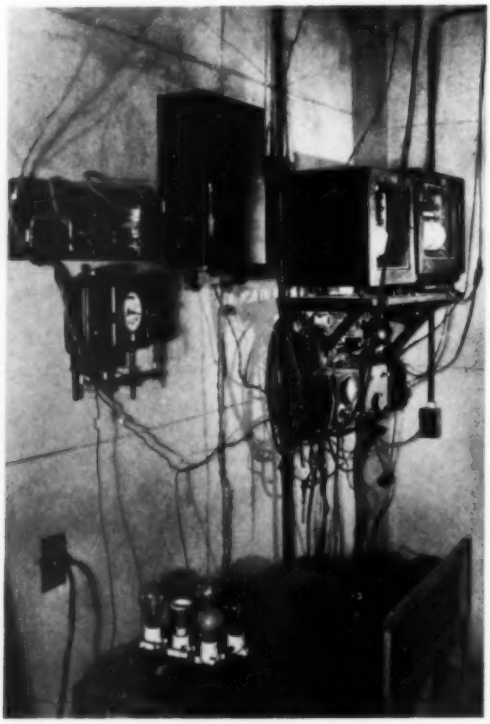

Fig. 7 Electrical recording instruments.

2 inches (5 cm.) from the end of the rod serves to keep the thermometer comfortably in position. The coil and rod are dipped and baked with three coatings of transparent bakelite, which is found to be the most satisfactory of any coatings tried. Leads from the thermometers leave the chamber by a

hole in the upper surface to be connected to the multiple bridge shown diagrammatically in figure 8. A switch arm 'S' swings over a series of mercury cups 'C,' by which in turn each of the thermometers is brought into the Wheatstone network with its appropriate balancing resistance. In the

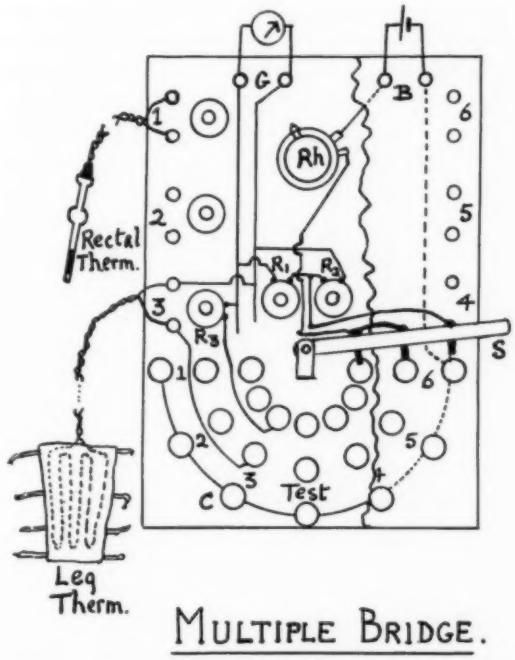

Fig. 8 Multiple bridge for body temperature measurements.

diagram, half of the bridge cover is shown cut away to reveal the essential connections to one of the thermometers, the leg thermometer, only. R_3 is the fixed coil balancing this thermometer; R_1 and R_2 are the 'ratio coils' of the bridge. The deflection of the galvanometer gives directly the temperature of the thermometer on reference to a calibration chart made when the thermometers are immersed in a metal con-

tainer in stirred constant temperature baths. In the central position the switch connects, instead of a thermometer and its balancing resistance, two fixed resistances, to give a 'test deflection.' Adjustment of this to a standard value by the rheostat Rh ensures accuracy of the readings. Current from a 1½ volt dry cell is sufficient to give a sensitivity of 3 cm. on the scale to a degree centigrade, which is ample for physiological experiments. The temperatures of all the four thermometers can be read in the space of less than a minute. In routine calorimeter experiments they are recorded every 5 minutes, though for the calculation of the direct heat the temperature at the beginning and end of a period are the only ones needed.

Electrical heat checks. The calculation of the total heat received by the apparatus requires a knowledge of the number of Calories per hour escaping through the insulation for each value of the gradient across it, and also of the number of Calories that are absorbed for each degree rise of the temperature of the wall—the hydrothermal equivalent of the apparatus. Both of these are found by a series of experiments in which energy of known amount is supplied in the chamber by an 'electrical man.' This is a wooden framework on which resistance wire is strung and which is covered with asbestos cloth to give an area and shape similar to that of a man. The energy supplied to this is read from a kilowatt-hour meter. (See in fig. 7 on the wall in the foreground.) The rotating disc in the meter carries a light silvered coverslip which, once per revolution, reflects the light of a lamp mounted above to a photronic cell, which through a Weston signal relay system, actuates a counter. Multiplication of the number of revolutions this counter records in a period by a factor gives the number of Calories supplied to the chamber. The meter has been calibrated against standard voltmeters and ammeters through the courtesy of the General Railway Signal Company of Rochester.

In the check experiments, the rate of water flow and of energy supply is adjusted until thermal equilibrium is

reached, so that in an hour period there is no recorded change of temperature of the wall, and thus no item of heat to be reckoned as entering or leaving the apparatus itself on that score. The heat entering the water, the air, and that lost by evaporation are calculated. The discrepancy between the total and the heat electrically supplied gives the heat that has escaped through the insulation of the calorimeter. The rate of the heat loss, or gain, is then plotted against the average gradient of the period, to give the curve used in future experiments to calculate the heat loss from the average gradient. Since, in the determinations of this curve, all the sources of heat measurement are present that occur when human calorimetery is studied, the deviation of the points from a smooth curve is a measure of the errors in the measurement of heat by the apparatus. The errors are found to be not greater than 1 or 2 Calories per hour. In practice, the temperature of the calorimeter room is set to such a value that the gradient of temperature across the insulation is small, and the item of heat lost or gained by this route is a small one.

For the determination of the hydrothermal equivalent, the experiment is continued after the electrical energy has been shut off. The records of the temperatures are then as in figure 9 (where the three charts are combined and scales for wall temperature, gradient and cooling of air are on the left-hand side, that of the water difference on the right). The rise of temperature of the flowing water does not fall immediately to zero, since the water continues to receive heat from the cooling chamber. A tangent drawn to the curve of falling wall temperature at a point such as that of the dotted arrows gives the rate of fall of temperature in degrees per minute. The values of the temperatures on the other curves allow the number of Calories per minute given off to be calculated. Division gives the hydrothermal equivalent. An example is given in table 1. The constancy of the results of the thirteen determinations given at the foot of the table is an indication of the accuracy of the heat measurement. Since in an ordinary

experiment the change of wall temperature is rarely more than 0.1°C., the item of the heat gain or loss of the glass chamber itself is known with considerable accuracy. The above method is considered much better than estimation of the hydrothermal equivalent from weight and specific heat of the chamber.

Residual analysis. It is necessary to know what change has occurred during a period in the carbon dioxide, oxygen and water vapor contents of the chamber. To this end, at the

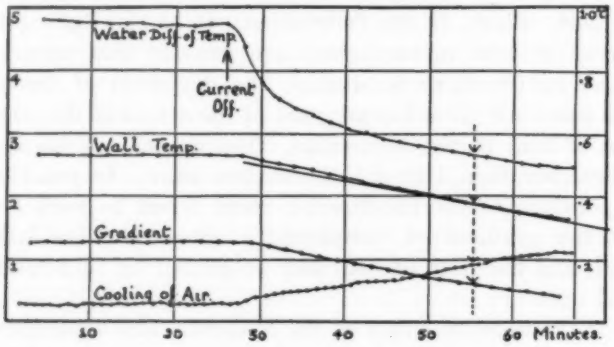

Fig. 9 Determination of hydrothermal equivalent.

beginning and end of each period, a sample of the circulating air is drawn off, passed through weighed absorbing U-tubes, and through a gas meter until 6 liters has passed, then back into the system. The temperature and pressure of the air in the chamber must also be known with accuracy, since a change of temperature of about 0.75°C. or a pressure of about 2.0 mm. produces a change of 1 liter in volume (total volume is about 560 liters). The pressure is, of course, read from a barometer, the temperature is taken as the average of three temperatures,[5] that of the ingoing, of the outgoing air, and of

[5] As this paper goes to press an improvement in recording the fluctuations of temperature of the chamber air has been made by employing several electrical resistance air thermometers in series.

a thermometer (seen in fig. 6), entering the top of the chamber above the subject.

Partition of the heat measured. Table 2 shows the relative magnitude of the various items that normally occur in the

TABLE 1

Calculation of hydrothermal equivalent

Rate of fall of wall temperature	0.0385°C./min.
Temperature difference of water	0.530°C.
Rate of flow of water	1.478 kilos./min.
Heat entering water	0.7848 Cals./min.
Cooling of air	2.11°C.
Rate of flow of air	34 liters/min.
Heat from air	0.0213 Cals./min.
Gradient across insulation	1.62 divs.
Heat into room	0.0133 Cals./min.
Total heat from wall	$= 0.7848 + 0.0133 - 0.0213$
	$= 0.7768$ Cals./min.
Hydrothermal equivalent	$= \frac{0.7768 \text{ Cals./min.}}{0.0385°\text{C/.min.}} = 20.2$ Cals./°C.

Results 22.1, 21.8, 22.3, 19.5, 23.0, 22.5, 22.1, 23.1, 21.9, 20.1, 23.5, 23.9, 20.2.
Average H.T.E. 22.1 ± 1.5 Cals./°C.

TABLE 2

Partition of heat measurement. Experiment July 19, 33

	PERIOD I	PERIOD II	UNIT
Weight of water flowing	90.45	92.20	Kilos.
Average rise of temperature	0.712	0.707	°C.
Heat into water	64.4	65.2	Cals.
Average gradient	2.11	2.13	Divs.
Heat into room	2.95	3.2	Cals.
Rise of temperature of wall	0.162	0.015	°C.
Heat into wall	3.8	0.35	Cals.
Average rise of temperature of air	— 1.97	— 1.97	°C.
Heat into air	— 1.55	— 1.55	Cals.
Total heat by radiation and convection	69.6	67.2	Cals.
Water evaporated	21.8	24.0	Gms.
Heat by evaporation	12.55	13.8	Cals.
Rise of rectal temperature	— 0.16	— 0.05	°C.
Heat stored by body	— 9.0	— 2.8	Cals.
Total direct heat	*73.15*	*78.2*	Cals.
Indirect heat	*74.9*	*78.7*	Cals.

total direct heat, of which some 80 per cent is usually in the flowing water. For the heat lost by evaporation, the figure 0.580 Calories per gram of water evaporated is used. The latent heat of vaporization of water at 20°C. is 585.84 Calories; at 30°C. 580.04 Calories. The value of 586 Calories, correct for 20°C., has been used by some workers. The evaporation of water from the human skin takes place at a temperature certainly above 30°C., the average skin temperature being about 33°C. The value 0.580 kilocalorie per gram of water evaporated is therefore used. The water collected in the acid bottles of the train may not truly represent the amount evaporated by the subject, since water may accumulate or be lost from the calorimeter walls or from the bed. It does accurately give, however, the heat removed from the calorimeter by evaporation for the calculation of the total heat received by the apparatus. There is, of course, a small correction due to cooling of the water vapor from the temperature of the skin to the temperature and relative humidity of the chamber.

The question of which figure should be used in the calculation, one of academic rather than practical interest, since the final differences are so small, is a complex one upon which we have encountered considerable difference of opinion. Without doubt, if we confine ourselves to consideration of the heat lost from the body surface by evaporation, the figure for the latent heat appropriate to the average skin temperature should be used. The balance of the heat lost by the body is through radiation, convection and conduction, but the amount of this portion of the heat loss is not accurately equal to the heat received and recorded by the absorbing system of the calorimeter, since the latter includes a small item of heat given up by the water vapor cooling to the final state of temperature and humidity in which it leaves the chamber. Thus in our partition of heat losses in table 2, the loss by evaporation is correct, but the loss by radiation and convection is slightly in error. (A calculation shows that the error in the case cited does not exceed 0.1 Calorie). Our total heat loss is also in error by the same amount, being the sum of an accurate parti-

tion to evaporation, and an inaccurate partition to radiation and convection.

Other workers, who are perhaps more interested in the accuracy of the total than in its component parts, retain the inaccurate partition to the radiation and convection and bring the total to supposed accuracy by altering the evaporation component, using the latent heat appropriate to chamber temperature instead of skin temperature and a correction for the cooling of the water lost by the body from body temperature to chamber temperature. Their total heat loss is then (supposedly) correct, but is composed of two components, neither of which can be accurately partitioned to evaporation or to radiation and convection. The total is not really accurate, since no account has been taken of the heat exchange in the expansion of the saturated vapor at chamber temperature to the relative humidity of the chamber—a not inconsiderable correction. Of the three quantities, the total heat loss and its two components, none can be calculated accurately without consideration of the relative humidity of the chamber. Our method has at least the virtue of giving one of the components, the loss of heat by evaporation from the skin, in an unequivocable way.

The heat stored or lost by the body of the subject in changing its average temperature may amount to a very large item, the uncertainty of our knowledge of which is discussed in the following paper.

Alcohol checks. Proof of the absence of gas leaks and of the accuracy of the calorimeter is sought by the normal method of burning 94 per cent ethyl alcohol in the chamber at frequent intervals between human experiments. Two small flames fed by asbestos wicks in a glass Y-piece, were found to be much steadier than a single flame of the same capacity. The burner is connected by rubber tubing to a regulating apparatus fixed to the outside of the calorimeter box. By the opening or closing of two stopcocks the alcohol is siphoned alternately from one or the other of two bottles supported on blocks of wood. At the end of an hour's period, by turning

the taps the supply is shifted to the other bottle, while the blocks beneath the first are removed so that it may be lowered, removed, weighed and replaced ready for a further period. In this way the weight of alcohol burned in the actual period of experiment may be directly found by weighing for a number of successive periods. The steadiness of the flames is ensured by keeping the level of alcohol constant by the insertion of thin cards under the bottles when needed.

In table 3 the results of all alcohol check periods made during a space of more than a year are given. Those periods in which there was an obvious leak, shown by the very poor value of the R.Q., are the only ones omitted. The close agreement between the averages of the direct heat from the calorimetry and the indirect heat from the amount of oxygen consumed, amounting to 0.17 per cent, shows that the systematic error in the heat measurement is very small.[6] The average of the differences between direct and indirect heats in individual hour periods is 2.35 Calories, or 2.9 per cent, and, as the equality of the totals shows, is as likely to be positive as negative. This 2.9 per cent and not the 0.17 per cent, is the correct average instrumental error of the apparatus in comparing direct and indirect heat. The average error in measurement of either direct or indirect heat alone must be less than this.

It is interesting to find that the systematic error' (percentage difference between totals) is exactly the same in this series as was given by Gephart and DuBois ('15) for the first series on human subjects run with the Sage calorimeter. Coincidently also the first report of alcohol checks with the Sage calorimeter included nineteen periods (Riche and Soderstrom, '15) as does the present report. The percentage errors found have not been calculated on quite the same basis in this report as in that of Riche and Soderstrom, but they are obviously very similar. For example, in the Sage calorimeter

[6] I.e., there is no tendency to give regularly either too low or too high a value for either measurement. The mode of the difference lies at + 0.142 Calorie on the side of the direct heat.

the average total error for oxygen in the alcohol checks was —1.69 per cent; in the present series it is —1.4 per cent. The average individual error for the difference between theoretical

TABLE 3

Alcohol heat checks

DATE	ALCOHOLISM CONSUMPTION IN GRAMS	$L.O_2$ THEORY	FOUND $L.O_2$	HEAT THEORY	HEAT FOUND		R.Q.
					Direct	Indirect	
1933							
May 4					82.4	81.4	0.672
May 9	12.75	18.12	18.47	88.7	87.8	89.6	0.628
	10.88	15.40	15.55	75.8	73.0	76.0	0.722
May 15	8.47	11.50	10.93	56.1	56.8	53.1	0.687
May 19	11.48	16.37	15.67	79.5	80.0	76.1	0.674
	10.76	15.40	15.09	76.2	72.0	73.3	0.671
	10.30	14.74	14.73	71.4	71.6	71.6	0.653
June 27	15.17	21.71	20.80	105.1	101.8	109.1	0.677
	13.21	18.90	18.64	91.6	86.5	90.4	0.650
July 14	13.31	19.02	18.44	92.3	88.2	89.6	0.662
	14.90	21.34	21.53	103.2	104.2	104.2	0.658
1934							
January 5	12.10	17.30	17.51	83.9	87.1	84.9	0.665
	13.06	18.70	18.70	90.4	89.9	91.5	0.654
January 15	11.32	16.20	15.48	78.5	76.4	75.1	0.659
	11.31	16.19	14.96	78.6	77.1	72.5	0.670
February 2	10.29	14.55	14.52	71.4	73.0	70.4	0.659
	11.23	15.88	16.24	77.9	77.2	78.8	0.656
May 25	11.98	16.23	15.92	78.6	78.9	77.2	0.670
	12.10	16.45	16.65	79.6	79.0	80.8	0.665
Averages	11.92	16.89	16.66	82.2	81.205	81.34	0.666
Totals		304.00	299.87	1478.8	1542.9	1545.6	
Difference			—1.4%		—1.3%	+0.17%	
Average of individual differences					±2.25%	±2.9%	±1.95%
Coefficient of variation			$\frac{\sigma \times 100}{\text{mean}}$		2.8%	3.6%	2.5%

heat and 'heat found' (presumably direct heat is meant) in the alcohol checks for the Sage calorimeter was 2.51 per cent without regard to sign; in the present series the same error is 2.25 per cent. The Sage workers do not give the average

individual difference between direct and indirect heat, with which the error of 2.9 per cent given above could be compared. The average respiratory quotient is exactly theoretical, but the average difference or scatter between the individual measurement and theory is $\pm$ 1.95 per cent.

Many investigators now prefer to see variations expressed as standard deviations from the mean and the coefficient of variation as a percentage which the standard deviation is of the mean. The latter are given also in table 3. These surely must be regarded as highly satisfactory.

Accuracy in human calorimetry. With human subjects two additional sources of error arise. The first, which has been mentioned, is that the indirect heat can be calculated from the oxygen consumption only by assuming values for the Calories per liter of oxygen that are accurate for a limited group of reactions taking place in the body, namely, the oxidations of fat, protein and carbohydrate. The indirect heat may then be in error by an unknown amount, which might conceivably be considerable in short periods.

It is still true that the calorimeter measures heat with an average error of less than 2.9 per cent, but what is measured is the heat eliminated from the subject. This is not, as it is with an alcohol flame, equal necessarily to the heat produced in the same period, as considerable amounts of heat may be stored or lost by rise or fall of body temperature. The body temperature can only be measured at a limited number of points (in the past one only, the rectal temperature has been used) and thus this item of the heat exchange has a large possible error. The result is that the average difference between direct and indirect heat in hourly periods is, with human subjects, of the order of 7 or 8 per cent when the rectal temperature alone is used. By using the surface temperatures also, in the manner explained in the following paper, the average error has been reduced to 5½ per cent.

The increased error cannot, however, fairly be considered as characteristic of the respiration calorimeter, the function of which is to measure the respiratory exchange and the heat

given off by a subject. Each of these types of measurement it makes with an average error not greater than 2.9 per cent.

SUMMARY

A respiration calorimeter is described in which the measurement of direct heat has many automatic features. The respiration chamber is a cylinder of Pyrex glass through which air circulates in a closed circuit including two interchangeable Benedict trains of absorbers. Oxygen is admitted automatically as it is consumed and is measured on a wet test meter. Cold water, brought initially to a constant temperature, passes through a grid of pyrex tubes to carry away most of the heat. The temperatures of inflowing and outflowing water and air, and the temperature of the glass wall are electrically measured and automatically recorded every minute. Water flow and air flow are recorded by meters of special design.

The calorimeter is not adiabatic, the escape of heat through the insulation around the walls being measured by the thermal gradient set up across it, which is electrically recorded by resistance thermometers. Calibration with electrical input of heat gives the heat flow for a given gradient and also the hydrothermal equivalent (22.1 Calories per degree).

In a series of alcohol checks, the totals of direct and indirect heats differ by 0.17 per cent, while the average difference in individual hour periods is 2.9 per cent. In human calorimetry, the average difference is more, but by the use of rectal and surface temperatures measured by resistance thermometers, it has been brought to 5.5 per cent.

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